IMPACT OF AIR POLLUTION ON ROOT COLONIZATION BY VAM FUNGI AND ROOT NODULATION ON BLACK GRAM

ABSTRACTS

THESIS

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IN

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BY

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INSTITUTE OF AGRICULTURE

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ABSTRACT

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The problem of environment pollution is increasing day by day and natural quality of the environment is regularly changing due to release of effluents and emissions from many different kinds of industries and other human activities.Since 90% of the plant weight is derived from the atmosphere, the quality of air is directly related to the growth and productivity of the plants.Air pollution has become now a new and important factor in agriculture and crop damages caused by air pollutants are being recognised in different parts of the world.

Air pollutants affect plants in various ways. They injure plant foilage, alter piant growth and yield and change the quality of the marketable plant products. The air pollutants also increase or decrease plant diseases caused by biotic pathogens. The phytotoxic air pollutants are, therefore, of great concern to agricultural scientists.

Root symbionts associated with air pollution-stressed plants are also likely to be influenced. Root nodule bacteria and VAM fungi which develop symbiotic associations with plants are known to be affected by impacts of air pollution on the plants.Some reports show that the sporulation and symbiotic associaion of VAM fungi are adversely influenced by air pollutants. According to some reports root nodulation is also suppressed. Whether or not the nodulated and mycorrhizal plants or plants with both types of root symbionts at their roots respond differently under air pollution stress,from those which are non-symbiotic is not fully established because this aspect of air pollution has not received adequate studies. The present study aimed to determine some of these effects of air pollutants and root symbionts on black gram in artificial treatment conditions. Sulphur dioxide, ozone and fly ash were used as air pollutants. A strain of *Rhizobium* sp. which nodulated black gram roots and *Glomus caledonicum* a VAM fungus were used in the study as root symbionts. Three-week-old plants were exposed to different concentrations of SO_2 (0.05 and 0.1 ppm) and O_3 (0.02-0.05-0.02 and 0.05-0.1-0.05 ppm) by placing the potted plants in exposure

chambers. The pots were exposed for 3h (SO_2) and 7h (O_3) on every alternate days upto 80 days. Fly ash was obtained from a coal fired thermal power plant, located at Kasimpur, Aligarh district, U.P. (India). The field soil and fly ash were mixed in requisite quantities to obtain different levels of fly ash (20, 40, 60, 80 and 100% v/v). The parameters considered were plant growth (lengths, fresh and dry weights of shoot and root), yield (number of pods per plant and number of seeds per pod), chlorophyll content of leaf (chlorophyll a, chlorophyll b, and total chlorophyll), nitrogen and phosphorus contents of shoot and root of black gram, root nodulation (number of nodules per root system and dry weight of nodules) and root colonization and sporulation of the VAM fungus.The results of the study are given below in brief.

Sulphur dioxide

Root symbionts (*Rhizobium* sp. and *G.caledonicum*)improved plant growth (lengths, fresh and dry weights of shoot and root)of black gram. Increase was highest in the presence of both the root symbionts. The increase caused by *Rhizobium* sp. alone (single inoculated-nodulated) was less than the increase caused by *G. caledonicum* (single inoculated- mycorrhizal).Plants exposed to either concentration of SO_2 exhibited suppressed growth irrespective of the treatment. The reduction was greater at 0.1 ppm than 0.05 ppm of SO_2. At both the concentrations, plants inoculated with both the root symbionts showed better growth than those inoculated with either of them or uninoculated plants.Yield of the plants of all treatments at both concentrations of SO_2 was reduced but the reductions in various treatments were not significant.

Leaf chlorophyll (chlorophyll a, chlorophyll b, total chlorophyll), seed protein and nitrogen and phosphorus contents of shoot and roots were highest in dual inoculated plants. Single inoculated plants with either of the two root symbionts also showed improved leaf chlorophyll and seed protein and nitrogen and phosphorus contents as compared to non-symbiotic plants. Exposure of the plants of various treatments to SO_2 concentrations caused reduction in leaf chlorophyll, seed protein and phosphorus and nitrogen contents of the plants. Higher concentration of SO_2 was more effective. Nodulated, mycorrhizal and

dual inoculated plants suffered comparatively less losses in these parameters than non-inoculated plants exposed to SO_2.

Root colonization and spore number of the VAM fungus on black gram was highest in dual inoculated plants. SO_2 exposures at both concentrations suppressed root colonization and spore number of the VAM fungus both in dual (*Rhizobium* sp. + *G. caledonicum*) or single inoculated (*G. caledonicum*) plants when compared to unexposed inoculated plants.

Root nodulation (nodule number per plant and dry weight of nodules) by *Rhizobium* sp. was greater in plants inoculated with both the root symbionts than plants inoculated with *Rhizobium* sp. alone.Exposures of plants to SO_2 concentrations suppressed root nodulation both in single and dual inoculated plants. The higher concentration was more suppressive.

OZONE

Inoculation of black gram with the root symbionts,*Rhizobium* sp. and *G.caledonicum* improved plant growth and yield.Plant growth and yield were suppressed when exposed intermittently to O_3 at 0.02-0.05-0.02 and 0.05-0.1-0.05 ppm. Suppression in growth and yield was less in symbiotic plants in comparison to non -symbiotic (uninoculated) plants.Dual inoculated black gram plants contained highest amounts of chlorophyll, seed protein, nitrogen and phosphorus.O_3 exposures caused suppressions in all the above mentioned parameters.The concentration 0.05-0.1-0.05 ppm was more effective than the other concentration.

Root colonization and spore production of the VAM fungus on black gram in dual inoculated plants were greater than plants inoculated with *G.caledonicum* alone. At both concentrations of O_3, root colonization and spore production of the VAM fungus were suppressed. Suppression was less in dual inoculated and mycorrhizal plants compared to uninoculated plants.

Root nodulation was significantly reduced at both the concentrations of O_3.Dual inoculated plants showed less reduction in

root nodulation than single inoculated (*Rhizobium* sp.) plants. Inhibition of a root nodulation was greater at 0.05-0.1-0.05 ppm. O_3 than 0.02-0.05-0.02 ppm.

Fly ash

Black gram plants grown in 20,40,60% fly ash showed a significant enhancement in plant growth and yield. The plants inoculated with both the root symbionts showed highest growth and yield. Fly ash at 80 and 100% caused a reduction in plant growth and yield irrespective of the presence or absence of the root symbionts. Lower levels of fly ash(20-60 %) enhanced leaf cholorophyll,and seed protein and nitrogen and phosphorus contents of shoot and root. At higher level (above 60%) fly ash caused significant suppressions in the above parameters .

At all the levels of fly ash, root colonization by the VAM fungus was suppressed but spore production increased at the lower levels of fly ash (20-40%).At 60% level,however,spore production decreased in most of the treatments.Higher level of fly ash (80 and 100%)caused reduction in spore production in all the treatments.

Significant increase occurred in nodulation at 20 and 40% levels of fly ash.From 60% onwards reduction was observed in root nodulation.

The study has shown that VAM fungus (*G. caledonicum*) and root nodule bacterium (*Rhizobium* sp.) by symbiotic relationship improved plant growth,yield and other considered parameters of black gram. SO_2 and O_3 were suppressive in general for plant growth and yield and other parameters determined.The losses suffered in all the considered parameters by the plants were greater in uninoculated plants i.e. in the absence of the root symbionts than in the presence of either or both the root symbionts. Presence of both the symbiont was most beneficial.. They provided partial protection to the plants against the adverse impacts of gaseous air pollutants though they also suffered suppressions.These attributes of the root symbionts can be agriculturally exploited in order to save the crop plants especially legumes from adverse effects of air pollutants.

Fly ash at lower levels was beneficial for the plants but higher levels were inhibitory.Root symbionts were also suppressed by the higher levels of fly ash. Accumulation of fly ash in soil beyond certain levels is harmful both for the crop plants like black gram and root symbionts,the microorganisms,useful for agriculture.

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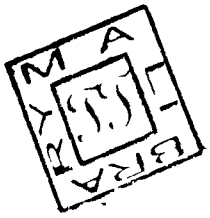

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CERTIFICATE

This is to certify that Miss **MADHU KULSHRESHTHA** has worked as a Research Scholar in the Institute of Agriculture under my guidance. Her thesis on **"IMPACT OF AIR POLLUTION ON ROOT COLONIZATION BY VAM FUNGI AND ROOT NODULATION ON BLACK GRAM"** is original and upto-date. She is allowed to submit this thesis for consideration of the award of degree of **DOCTOR OF PHILOSOPHY IN AGRICULTURE** (Plant Pathology).

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INTRODUCTION

Air pollution has become a serious environmental problem in different parts of the world. Air pollutants originating from various kinds of industries fall into two categories i.e., gaseous and particulate. The important gaseous air pollutants are sulphur dioxide (SO_2), oxides of nitrogen (NO_X), carbon monoxide (CO), ammonia (NH_3), chlorine (Cl_2), ethylene (C_6H_6), hydrogen fluoride (HF), ozone (O_3) and peroxyacetyle nitrate (PAN). Some air pollutants such as SO_2, CO, NO_X, NH_3, HF are called primary air pollutants and some such as O_3 and PAN as secondary air pollutants depending upon their origin (Wood, 1968). Primary air pollutants directly originate from their source while secondary air pollutants are formed by reaction of primary air pollutants with other environmental factors.

Certain ranges of environmental factors and air quality are necessary for the proper growth and plant health, as over 90% biomass of green plants is derived from atmosphere. Plant growth and yield are adversely affected, directly or indirectly by air pollution (Mudd and Kozlowski, 1975, Heck *et al.*, 1982). Air pollutants induce injuries of various kinds in a number of agricultural and horticultural crops. Adverse effects of air pollution on agricultural crops are being assessed in different parts of the world. The Environmental Protection Agency (EPA), in U.S.A. estimated that in 1976 annual losses to agriculture production caused by poor air quality was around 2.9 billion dollars.Yield losses have been found to be caused by air pollution in a large number of crop plants including soybean, peanut, cotton, tobacco, vegetable crops, ornamentals etc.

Direct injury to leaf tissue or interference in biochemical reactions in leaves are main effects of gaseous air pollutants after their entry through stomata (Pell, 1979). Particulate air pollutants fall and deposit on the leaf surface forming a thin encrustation on the leaf surface which affects transpiration and transmission of solar radiation (Darley, 1966). Sulphuric acid or nitric acid formed by gaseous air pollutants SO_2 and NO_2 respectively by reaction with water, either directly injure the plant parts or indirectly through soil harm the root system. Air pollutants affecting physiology and biochemistry of the plant, induce visible symptoms like chlorosis, necrosis, early senescence, stunting etc. (Heagle, 1973, 1982; Agrios, 1988).

Mycorrhiza is a term which designates a symbiotic relationship between fungi and plant roots. There are two main types of Mycorrhizae viz., ectonmycorrhizae and endomycorrhizae.Ectomycorrhizae belongs to the other Agaricales of the class Basidiomycetes. They modify the plant roots and absorbent hairs of roots are lost. The ectomycorrhizal fungus surrounds the roots and forms a mantle of mycelium. The external hyphae originating from the mantle explore the soil and help in absorption of nutrients and water, while the internal hyphae make a close contact with the roots.

Endomycorrhizae belong to the order Endogonales of class Zygomycetes. Endomycorrhizae are characterized by intercellular infection within the root and absence of any organized fungal growth on the root surface. They may be formed by non-septate fungi referred to as vesicular- arbuscular mycorrhizae.

Vesicular-arbuscular mycorrhizal fungi which have attracted greater attention of agricultural scientists are intercellular within the roots and form vesicles and arbuscules of the endophytic phase. Inside the root hyphae are limited to only root cortex and grow usually intracellularly where thick-walled hypae with angular projections give rise to fine, thin-walled, irregular branches, the arbuscules in inner cortex and thick- walled spores and the vesicles in the outer cortex.

VA mycorrhizae are agriculturally more important than other mycorrhizae. The most important role of VAM fungus is to absorb nutrients from the soil and transfer them to their hosts. The exchange of nutrients between host cells and fungus occurs through arbuscules whose function is like that of haustoria. Mycorrhizal fungi in symbiotic relationship with plant roots help plants in acquiring mineral nutrients from the soil, especially immobile elements such as P, Zn and Cu but also more mobile ions such as S, Ca, K, Fe, Mg, Mn, Cl, Br and N (Cooper and Tinker, 1978; Tinker, 1984). Mycorrhizae improve the phosphorus nutrition of a host particularly in low fertility soil through exploration of the soil by the external hyphae beyond the root hairs and phosphorus depletion zone (Gray and Gerdemann, 1969). Increased P uptake in mycorrhizal legumes stimulates nitrogen fixation by *Rhizobium*, thus indirectly causing an increase of nitrogen concentration in the host (Carling *et al.*, 1978; Schenk and Hinson, 1973). Mycorrhizal fungi have been shown to tap organic and inorganic phosphorus sources in soil which are normally unavailable to non-mycorrhizal plants (Powell, 1979).

Plant growth is enhanced by the mycorrhizal fungi due to increase in the efficiency of mineral uptake. Increased water uptake by mycorrhizal fungi alter the plant physiology to reduce stress response to soil drought (Parke *et al.*, 1983, Safir and Nelson, 1985). Mycorrhizal fungi also reduce plant response to other soil stresses such as high salt levels, toxicities associated with mine spoils or land fills and heavy metals (Tinker, 1984). Disease response to plant pathogens causing morphological or physiological changes in the plants is reduced by mycorrhizae (Dehne, 1982). VAM infection counteracts adverse soil factors. They alleviate heavy metal toxicity (Dueck *et al.*, 1986) and increase the tolerance of crops to high acidity and temperature (Poss *et al.*, 1985; Pond *et al.*, 1984). Mycorrhizal fungi are known to alter soil texture by increasing the extent of soil particle aggregation (Sutton and Sheppard, 1976). One of the major changes in mycorrhizal plants is reduced membrane permeability primarily due to increased P nutrition (Graham *et al.*, 1981; Ratnayake *et al.*, 1978). The decreased membrane permeability affects the quality and quantity of root exudation (Schwab *et al.*, 1983) which in turn induce a significant response in the rhizosphere microflora and microfauna.

VAM fungi may serve as a tool for improving the growth of agricultural and horticultural plants (Tinker, 1978; Gianinazzi *et al.*, 1989; 1990a, b). The symbiotic systems of these fungi can also be exploited to save the costly phosphatic fertilizers (Abott and Robson, 1982a, b).Their importance in natural ecosystem (Koske, 1981) and in revegetation of the disturbed lands has also been recognized (Reeves *et al.*, 1979).

Legumes can form two types of symbiotic relationship with microorganisms: one with nitrogen-fixing species of *Rhizobium* and *Bradhyrhizobium*, the other with vesicular-arbuscular mycorrhizal (VAM) fungi, concerned mainly with the uptake of phosphorus by the plants. Glasshouse experiments have demonstrated that legumes inoculated with both types of microorganisms grow and nodulate better and have higher nitrogenase activity and phosphorus content than plants that are uninoculated or inoculated with either root nodule bacteria or mycorrhizal fungi separately (Crush, 1974, Powell, 1976). Seed yield, shoot weight and percentage of P and N of nodulated soybean plants grown in P deficient field soil were increased by inoculation with *Glomus fasciculatus* (Bagyaraj *et al.*, 1979a). Similar effects on growth of soybean, bean, alfalfa and peanut under glasshouse conditions have been reported (Daft and El-Giahmi, 1975).

VAM fungi may be influenced by a wide range of environmental and edaphic factors. Greater plant growth enhancement due to mycorrhizae occur in sterile soil than in non-sterile soil (Gerdemann, 1968). Mycorrhizal development and plant growth response decreases with an increase in soil fertility (Hayman and Mosse, 1972; Khan, 1975). The nutrient (N or P) rich soils contain fewer spores of VAM fungi,which can be recovered from field soil of several agricultural crops than soils which are nutrient deficient (Hayman, 1970; Hayman and Mosse, 1972).

Effect of air pollution on roots and mycorrhizae has only recently received some attention of researchers. The ectomycorrhizae have been shown to alter some of the effects of air pollutants through promotion of shoot and root growth (Carney *et al.*, 1978; Garrett *et al.*, 1982; Mahoney *et al.*, 1985). Interactions between O_3 and simulated acid rain and ectomycorrhizal treatment and soil regime had significant effects (Keane and Manning, 1988). Reich *et al.*,(1985) observed the adverse effect of O_3, SO_2 and acid precipitation on mycorrhizae of red oak (*Quercus rubra* L.). VAM fungi have gained very little attention in relation to gaseous and particulate air pollutants.

Microorganisms, parasitic or non-parasitic associated with plants growing under air pollution stress are likely to be affected directly or indirectly. Symbiotic nitrogen fixation by root nodule bacteria in leguminous crops is a very significant natural biological process and important for nitrogen economy of soil. Air pollutants affect nitrogen fixation and root nodulation (Tigney and Blum, 1973; Shriner, 1978). It has been suggested that inhibition of N_2-fixation results from reduction in the nodulation and suppression in bacterial population. These effects are caused through the impact of air pollutants on plants. Ozone (O_3) , SO_2 and particulate matters are reported to suppress N_2-fixation by the species of *Rhizobium* (Tigney and Blum, 1973; Shriner and Johnston, 1981). Some workers have observed reduced root nodulation, nitrogen fixation, and or leghaemoglobin content of leguminous plants following 1 or 2 acute O_3 exposures in greenhouse or controlled environment chambers (Blum and Heck, 1980; Blum and Tigney, 1977). Root nodulation by *Rhizobium* in kidney beans grown in greenhouse or outdoor plots and soybeans grown in greenhouse (Shriner, 1978; Waldron, 1978) was reduced by sulphuric acid rain of pH 3.2. Root nodulation was inhibited by SO_2 and O_3 exposures at 0.1 ppm and 0.2 ppm in soybean (Singh, 1993), mungbean and chickpea (Jahan, 1983) and chickpea (Abbas, 1994).

Determining the response of vesicular-arbuscular mycorrhizal fungi (VAM) and root-nodule bacteria on leguminous plants growing under specific pollution stresses is the theme of the present work. Black gram (*Vigna mungo*) a pulse crop was selected as test crop in the present study because it is one of the important pulse crops grown in India, which are sources of protein to vast vegetarian population of the country; can be sown in two season i.e., June-July (rainy season) and October-November (Autumn); and most importantly the crop develops symbiotic relationships with root nodule bacteria and VAM fungi.

The system with components-host (*Vigna mungo*), symbionts-VAM fungus, (*Glomus caledonicum*) and nodule forming bacterium (*Rhizobium* sp.) has been used as a model to ascertain the impact of air pollution stress on the various components. Three major air pollutants SO_2 , O_3 and fly ash were used to assess the impact. Thesis is divided into introduction, literature review, materials and methods, results, discussion and summary. References cited in the different sections are listed at the end.

LITERATURE REVIEW

Air pollution has entered as a new factor in agriculture and crop damages caused by air pollutants are now recognised in different parts of the world. A number of chemical substances present in the air surrounding various kinds of industries are toxic to plants. Ozone (O_3), sulphur dioxide (SO_2), nitrogen oxide (NO_2)and ammonia (NH_3) are four most important and phytotoxic air pollutants (Heagle, 1973, 1982).

Air pollutants affect plants in various ways. They injure plant foilage significantly,alter their growth and yield and change the quality of the marketable plant products. Ozone alone or in combination with SO_2 and /or NO_2 causes crop losses upto 90% in some cases (Heck *et al.*, 1982). The air pollutants also increase or decrease plant diseases caused by biotic pathogens (Heagle, 1973, 1982). The phytotoxic air pollutants are ,therefore ,of great concern to agricultural scientists.

Air pollutants on the basis of their physical appearance are grouped into two categories - gaseous and particulate (Wood, 1968). O_3, PAN, SO_2, Cl_2, C_6H_6, HF, H_2S, NO_X etc. are common gaseous air pollutants. Coal dust, fly ash, cement dust, soil dust particles etc. are major particulate air pollutants. Primary air pollutants like NO_X , SO_2 when come in contact with the water in atmosphere and atmospheric precipitation, are converted into acids and fall down. This condition of environmental pollution is called `acid rain' (Liken and Barman, 1974). Acid rain is an acute and severe air pollution problem in developed countries, while the particulate air pollutants are major problem in developing countries (Das, 1986). In India, sulphur dioxide and fly ash are most prevalent air pollutants as both are emitted from coal-burning which is abundantly used as energy source to run the industries. Concentrations of SO_2 for industrial areas has been recommended as 0.042 ug m^{-3} by the Central Pollution Control Board, India. But in many areas the concentration usually exceeds the prescribed safe limit. The plants may suffer damages at 0.05- 0.15 ppm SO_2 or beyond (Guderian and Stratmann, 1968).

Studies on plant diseases caused by air pollutants began in 19th century (Heagle, 1973, 1982). The extent and nature of injury or damage caused by air

pollutants are determined by genetic and environmental factors of plant, as well as by concentration and exposure duration to the pollutants (Heagle, 1973, 1982). Air pollutants induce visible symptoms like chlorosis, necrosis, early senescence, stunting and several other symptoms depending upon type of air pollutant and plant involved (Darley and Middleton, 1966; Brandt and Heck, 1968; Barret and Benedict, 1970). The existing information on some aspects of effects of some specific and important pollutants on plants is reviewed herewith.

Sulphur dioxide (SO_2)

Sulphur dioxide, one of the most important air pollutants that cause damage to plants, is emitted to atmosphere mainly through combustion of fossil fuels. Burning of coal and coal products, refining and utilization of petroleum and natural gas, manufacturing and industrial utilization of sulphuric acid and sulphur, smelting and refining of ores, especially of copper, zinc, lead and nickel are the sources of SO_2 emissions. However, the combustion of coal is the major source of atmospheric SO_2. According to Wood (1968) coal-burning power plants represent the most important single source of SO_2. The amount of SO_2 emitted through coal burning depends upon the sulphur content of the coal which varies from 1-6% of the total weight. The concentrations of SO_2 at ground levels depends upon the amount and concentration of emission, distance from the source and meteorological and topographical conditions. In general, SO_2 concentration decreases rapidly with the distance from the point source and with increased air movement. SO_2 concentration near point sources, such as coal-burning power plants and smelters, with little or no pollution control equipment, has been found to be as high as 1 to 3 ppm. SO_2 concentration in large urban areas ranges from 0.05 to 0.04 ppm (Heagle, 1973, 1982).

The mode of entry of SO_2 in plants and the overall mechanism of damage of leaf tissue have been examined by various workers. SO_2 enters the leaves through stomata and reacts with water in mesophyll tissue to produce sulphite ions, which is slowly oxidised to sulphate ions. The sulphate ions may be utilized by the plant as a nutritional sulphur and converted to organic form (Thomas *et al.*, 1944). Sulphite and sulphate ions, however, in excess amounts become toxic to plant cells. Sulphite ions are much more toxic than sulphate ions (Thomas *et al.*, 1943).

SO_2 causes two general types of symptoms or injuries referred to as chronic and acute. Accumulation of sulphite ions causes appearance of both of these symptoms. General chlorotic appearance of the leaf, mild chlorosis, yellowing of leaf, silvering or bronzing of the under surface of the leaves are considered as chronic symptoms. Some plants show white types of chronic markings and red, brown or black patches on the leaves (Barrett and Benedict, 1970). Absorption of lethal quantities of SO_2 leads to acute symptoms on plants. Tissues of marginal or inter- costal areas of leaves become dead. First they become greyish green water-soaked in appearance but later on drying become bleached ivory in colour. The dead or necrotic areas may fall out and the leaves give a very ragged appearance. When major portion of the leaf is injured, it is shed by formation of an abscission layer at the base of the petiole (Barret and Benedict, 1970). At low concentration, SO_2 causes chlorosis of leaves without formation of necrotic lesions and veins characteristically remain green (Darley and Middleton, 1966; Agrios, 1978, 1988).

Sulphur dioxide affects both physiological and biochemical processes of plants. Photosynthesis of affected plants is generally reduced, but transpiration and dark respiration are increased. Such effects have been observed both in short and long term exposures of plants (Black and Unsworth, 1979; McLaughlin *et al.*, 1979; Takemoto and Noble, 1982; Saxe, 1983). SO_2 effects on enzyme systems and metabolic processes are related to SO_2 concentration, plant species, plant age and environment. In some cases, enzyme activity is increased by exposure of the plants to low level of SO_2 and decreased by higher concentration (Horsman and Wellburn, 1977; Soldatini and Ziegler, 1979; Wyss and Brunold, 1980; Piere and Quieroz, 1982; Tanak *et al.*, 1982). SO_2 affects plant metabolism in a variety of ways. It stimulates phosphorus metabolism (Plesnicar, 1983), and reduces foliar chlorophyll concentration (Pandey and Rao, 1978; Lauernorth and Dodd, 1981). Carbohydrate levels are increased by low and decreased by higher concentrations of SO_2 (Kozoil and Jordan, 1978).

Adverse effects of SO_2 on physiology and biochemistry of plants significantly influence their growth, development and productivity. These effects of SO_2 on crop plants have been shown in studies conducted both in glasshouse and ambient conditions. Pandey and Rao (1978) exposing wheat plants to SO_2 recorded reduction in root and shoot lengths, number and area of leaves per plant,

biomass, productivity and number of grains per spike. No chlorosis or necrosis of leaves were, however, observed. Sprugel *et al.* (1980) found significant reduction in yield of soybean plants exposed to 0.09 to 0.79 ppm in an open air fumigation chamber. Leaf injury was not frequently observed. Slight increase in biomass was, however, reported in alfalfa exposed continuously to 0.036 ppm concentration of SO_2 (Lockyer and Cowling, 1981). Exposure of tomato plants to 0.12 ppm concentration of SO_2 for 72 h/week for 5 or 10 weeks, caused no effects on fruit yield and other soluble and total solid contents but slight decrease in ascorbic acid of ripe fruits (Lotstein *et al.*, 1983). In *Vigna sinensis* exposed to 0.12, 0.250.5 ppm of SO_2, bifacial necrotic lesions appeared on the middle and lower leaves exposed to 0.25 and 0.5 ppm SO_2. A slight stimulation in plant height, root and shoots lengths was observed in 0.12 ppm SO_2 exposure in early stages of plant growth (Kumar and Singh, 1986). Exposure of intact pinto beans and those with cotyledons removed immediately after germination to 0.15, 0.25 and 0.50 ml/lit SO_2 at an identical dose (0.50 ml/lit/h/ day) for 4 weeks reduced leaf area, shoot and root dry weight and increased shoot/root ratio and specific leaf areas in all exposed plants (Temple *et al.*, 1985). Bytnerowicz *et al.* (1987) examined impact of SO_2 on winter wheat in open top chamber . After 22 days of exposure decrease in lower buffering capacity, increase in total sulphur content and increased injury, particularly at higher SO_2 concentration occurred.Broadbean *(Vicia faba* L.) crops exposed to SO_2 concentrations 165 ug m^{-3}, 62 ug m^{-3}, 74 ug m^{-3} in an open air field exposure system showed strongly affected leaf area development during the pod filling. Sulphur content was strongly elevated in the leaves and pods of the fumigated plants and the Ca content of the leaves was reduced by SO_2 (Kropff *et al.*, 1989). When soybean (*Glycine max),* maize (*Zea mays*), peanut (*Arachis hypogaea)* and navy bean *(Phaselous vulgaris)* were exposed to SO_2 for 8 h/day, the gas had little effect on soybean, accelerated more kernel ripening in maize, reduced the number of mature pods and kernels in peanut and reduced the bean weight in navy beans (Murray and Wilson, 1990). Adaros *et al.* (1991) found that barley was not significantly affected while the yield of wheat was decreased when both were exposed to 9-63 ug m^{-3} SO_2 for 24 h daily.

The reproductive development of rape seed *(Brassica napus)* was found to be significantly reduced by exposure to SO_2 (Bosac *et al.*, 1993). When the seeds of chickpea and lentil were exposed to SO_2 at 0.1 ppm and 0.2 ppm, seed

germination was found to be inhibited and the post-emergence mortality of the seedlings occurred. Chickpea was more sensitive than lentil to the exposures at germination stage. But after emergence the seedlings of lentil exhibited more sensitivity than chickpea. When plants were exposed to SO_2 at both the concentrations, reduction in plant growth, yield, leaf pigment, seed protein, number of stomata, number and length of trichome hydathodes occurred (Singh, 1989). Khan (1989) and Khan and Khan (1993) found chlorosis and small dot like patches, yellow to grey in colour on the tomato leaves exposed to SO_2. There was reduction in plant growth, yield, number and size of stomata,chlorophyll and carotenoid contents,and number of fruits/plant. Plant growth of cucumber was observed to be reduced, when exposed to SO_2 at 0.1 and 0.2 ppm for 3 days in a week in artificial exposure chamber (Pasha, 1991). The growth and yield of soybean plants was found to reduced when exposed to SO_2 at 0.1 ppm and 0.2 ppm for 3 days in a week in artificial exposure (Singh, 1993).

Ozone (O_3)

Ozone is most important air pollutant of photochemical oxidant. Automobile exhausts and other internal combustion engines are important sources of ozone. Incompletely burned hydrocarbons and NO_2 are released into the atmosphere by the automobile exhaust. In the presence of UV light, this NO_2 react with oxygen and forms O_3 and NO. Ozone may react with NO to form the original compound. But in the presence of unburned hydrocarbons the NO reacts with this hydrocarbons instead of O_3 and O_3 is released in the atmosphere (Agrios, 1978, 1988). O_3 concentration at ground level is generally less than 0.03 ppm.

Ozone is injurious to plant leaves exposed for even a few hours at concentration of 0.1 ppm to 0.5 ppm. Ozone enters leaves through stomata and causes injury to palisade parenchyma and other cells of leaves by disrupting the cell membrane. Affected cells near stomata collapse and die and white (bleached) necrotic flecks appear, first on the upper side and later on both the leaf surfaces. The colour of the affected leaves varies from light tan to red or almost black, depending upon the plant involved. Some plants such as citrus, grapes and pines show premature leaf fall (Darley and Middleton, 1966; Agrios, 1978, 1988). Epidermal cells remain uninjured while the palisade cells and spongy mesophyll become injured. Many injured cells remain alive but chloroplast is disrupted and the chlorophyll amount is reduced significantly (Hill *et al.*, 1961).

Ozone affects the physiological functions of the plants. Todd (1958) and Todd and Probost (1963) measured the effect of ozone at 4 ppm for 40 min on photosynthesis and found that development of symptoms were associated with inhibition of CO_2 fixation. Net photosynthesis decreased when plants were exposed to 0.06 ppm concentration of ozone for 1 h (Hill and Littlefield, 1969). In pintobean net photosynthesis initially decreased and total adenylate concentration increased after 3 h exposure to injurious concentration of O_3 (Pell and Brennan,1973). Net photosynthesis, however, returned to normal within 24 h. A significant increase in respiration of pinto bean leaves when exposed to 4 ppm for 40 min, was reported by Todd (1958). During the first hour after ozone exposure at 0.7 ppm, the respiration was inhibited before the visible symptoms appeared; the respiration increased only later, when visible symptoms had appeared (Macdowall, 1965). Hill and Littlefield (1969) observed decrease in the rate of transpiration at 0.06 ppm concentration exposure for 1 h. The sugar and starch content of the ash root decreased when exposed to 0.5 ppm O_3 for 8 h (Jensen, 1981). At 0.1 ppm O_3 mineral content except the Na increased in ladino clover.

Ozone is reported to cause various types of damages in a number of economically important crops such as soybean, potato, cotton, pepper, sunflower, clover, tomato, chickpea, lentil, mungbean etc. Exposure of seedlings of sensitive tomato cultivars to 0.4 ppm of O_3 for 2 h, repeated for 6 time caused 57% reduction in growth, when treated seedlings were transplanted in field (Henderson and Reinert, 1979). Pell *et al.* (1980) observed a decrease in tuber weight and total solids, but increase in reducing sugars in potato, exposed at several growth stages to 0.2 ppm O_3 for 3 h in glasshouse. Blum *et al.* (1983a) recorded 14 and 27% reduction in clover regrowth during second year at when exposed to 0.03, 0.06 and 0.09 ppm O_3 for 7 h/day in the field conditions for 2 years. At 0.05, 0.10 and 0.15 ppm O_3, the maximum root reduction (42%) and shoot reduction (24%) in clover were observed when exposed for 4 h/day for 6 days, 32 days after seedling (Blum *et al.*, 1983b). Exposure of soybean to 0.022 ppm and 0.112 ppm O_3 for 7 h/day in the field, caused a reduction in yield and oil content by 39% and 126% respectively, while protein content was not affected (Greenwald and Endress, 1984). Heggestad *et al.*(1985) showed a 5% reduction in the yield by exposing soybean plants to O_3 in open top chambers. Kats *et al.* (1986) exposed 3 cultivars of rice, M7, M9 and S201 in open top chambers to ozone and recorded a reduction in seed size and seed sterility in those plants which produced more

panicles. Heggested *et al.* (1987) exposed tomato cultivars to 0.011, 0.059, 0.118, 0.235 and 0.468 ppm in open field chambers with non-filtered (NF) air and 0.005, 0.113 and 0.466 ppm with charcoal filtered (CF) air for 57 days at 5 h/day and 5 days/week. A decrease in ripe fruit yield by 16% in NF compared with CF air was recorded. Khan (1989) exposed tomato plants to 0.2 ppm O_3 and recorded chlorosis of leaves and reduction in fresh and dry weights of shoot and root and number of fruits per plant. Soybean plants exposed to O_3 showed reduction in seed and growth by 7.9 to 18.6% (King and Nelson, 1987; Heggested, 1988). Ambient levels of O_3 caused little effect on vegetative growth or yields of four field grown tomato cultivars when exposed in open top chambers to charcoal filtered (CF), non-filtered (NF), but NF plus 1.5 times ambient O_3 concentrations reduced yields from 17% in C "UC204C" to 54% for cv. "Hybrid 31" (Temple, 1990). Chloroplast dimensions, photosynthesis capacity and nitrogen content of leaves decreased at high levels of O_3 exposure resulting in accelerated leaf senescence (Miyake *et al.*, 1989; Held *et al.*,1991). Adaros *et al.* (1991) observed a reduction in yield of wheat on exposure to O_3 at 6 to 44 ug m^{-3} for 24 h as compared to control treatment seven hour exposure to O_3 at 38, 45 and 50 nl/l (NF 18, NF 25 and NF 30 treatments) caused 26-42% reductions in the yield of *Phaselous vulgaris*. The leaves of such plants developed extensive symptoms of O_3 injury and were prematurely abscised from the plants. The reduction in yield was due to decrease in the numbers of seed/pod and the weight of individual seeds (Sanders *et al.*, 1992). Inhibition of seed germination of chickpea and lentil occurred by exposing pots to 0.1 and 0.2 ppm O_3. There was also reduction in plant growth, and number of flowers and fruits per plant at both the levels of O_3 (Singh, 1989). The length, fresh and dry weights of root and shoot, chlorophyll and nitrogen contents and seed protein were found to be decreased in soybean plants exposed to O_3at 0.1 and 0.2 ppm (Singh, 1993). The length, fresh and dry weights of shoot and root, number of pods/plant, chlorophyll, nitrogen and phosporus contents of plants and seed protein were reduced by 0.1 and 0.2 ppm O_3 in mungbean and chickpea plants (Jahan, 1993).

Particulate air pollutants

Coal dust, fly ash, lime dust, cement dust and soil dust particles etc. are the major particulate air pollutants. Production of coal, cement, combustion of coal,

gasoline, lime kiln operation, soil erosion, agricultural burning and wrong agricultural practices, volcanic erruptions, transportation and construction etc. are the sources of particulate air pollutants. Particulate matters settle on exposed plant parts, mainly foliage and on soil. Chlorosis, necrosis and death of the tissues may occur due to heavy deposition of the particles (Darley and Middleton, 1966; Heck *et al.*, 1970). The reduction in quality of vegetative and fruits occur by high particulate emission from the different sources (Heck *et al.*, 1970).

A reduction in transpiration rate, chlorophyll content and productivity of the wheat plants due to cement dust pollution was observed by Singh and Rao (1981). Colwill *et al.* (1979) observed a poor growth of the plants grown on roadside with highly busy traffic, where particulate matters were deposited on the leaves. The dusts of varying origin interfere with stomatal functioning mostly by filling and blocking the stomatal aperture (Ricks and Williams, 1974; Fluckiger *et al.*, 1978, 1979). Increase in leaf temperature (Eller, 1977; Fluckiger *et al.*, 1978) and transpiration (Beasley, 1942; Eveling, 1969), reduction in photosynthesis (Darley, 1966) and increase in the uptake of gaseous air pollutants (Ricks and Williams, 1974) occur. All these effects lead to poor growth of the plants.

Fly ash comprises finely divided particles of ash entrained in fuel gases arising from combustion of coal. Size of fly ash particles may vary from 0.02 μ to over 30 μ. It contains incompletely burned coal and the carbon content of fly ash may vary from 5-20% although some samples may contain as high as 50%. A large number of minerals such as SO_2, Al_2O_3, Fe_2O_3, CaO, MgO, SO_3, K_2O+N_2O,P_2O_5, SnO_2 and traces of Ni, Be, V, Hg, Se, Mn may occur in fly ash (Bhatia, 1978). Kamath (1979) by using instrumental neutron activation analysis, determined the concentrations of 17 elements in coal and corresponding fly ash (Na, K, La, Ce, Hg, Tb, Th, Cr, HF) collected from stack precipitators of coal-based power plants.

Fly ash is a fairly stable pollutant and accumulates in the soil through deposition on soil surfaces. Fly ash amendment of soil may also affect plant growth and their productivity. Some recent studies showed that plant growth of crops in the soils amended with fly ash is greatly influenced. Bhatia (1978) studied the growth pattern of *Hordeum vulgare* and *Portulaca* sp. by growing them in pots filled with fly ash (pH 8.8) and garden soil mixture in the ratio of 0:100, 5:95, 10:90, 25:75, 50:50 and 100:0. The control plants were grown in

garden soil only. The root and shoot biomass of these plants showed improvement upto 25% fly ash, but the best growth was attained at 5% fly ash. Pawar and Dubey (1982) studied the growth of wheat plants as affected by 5, 10, 20, 30 and 40% (W/W) fly ash in black cotton soil. They noted increase in plant height, dry matter production and photosynthetic pigments with 20% fly ash but at higher percentages, the plant growth was retarded. Mishra and Shukla (1986 b) treated plants of maize and soybean with fly ash, waste product of coal fired electric generating plants, at rate of 2, 4 and 8 g/m^3 day^{-1} for 30 consecutive days between 15 and 46 days of plant age. At the lower two rates, both crops showed an increase in plant height, metabolic rate, content of photosynthetic pigment and all dry weight fractions measured. This response was due to correction of boron deficiencies by fly ash deposit. The highest rate caused reduction in all parameters. Khan (1989) observed beneficial effects of fly ash on tomato plants upto 70% level, when different level of fly ash (0,10,20,30,40,50,60,70, 80,90,100%) were added to soil. Gradual increase in length , fresh and dry weights and chlorophyll content upto 70% and decrease at 80,90 and 100% fly ash levels were observed .

Fly ash in soil increased the porosity, water holding capacity, conductivity,organic matter, sulphate and biocarbonate, carbonate contents, but lowered the soil pH (upto 8). Increasing the level of fly ash i.e. 60% onwards upto 90% resulted in suppression of leaf pigments, seed protein, stomatal number and size in chickpea and lentil. The number of trichome hydathodes, however, increased in chickpea (Singh, 1989).

Analysis of fly ash from the Thermal Power Plant, Kasimpur, Aligarh, was done by Pasha (1991). Total organic carbon and total nitrogen were 0.07%, 0.05% respectively. The total metal elements analysed were Pb (27.56 ppm), Ni (06.90 ppm), Mn (22.80 ppm), Co (3.82 ppm), B (21.71 ppm), Cu (01.52 ppm), K (722.20 ppm), Cr (13.91 ppm), Cd (0.24 ppm), Zn (03.04 ppm), Fe (02.43 ppm). The concentrations of K, Pb, Mn and B were higher than other metal elements. He also studied effect of fly ash amended soil on plant growth of cucumber. Best growth of cucumber plants was seen in 10 and 25% fly ash. Increase in chlorophyll content was observed in soil amended upto 50% fly ash. Singh (1993) observed that increasing the level of fly ash i.e. 50% onwards upto 100% resulted in suppression of plant growth, seed protein, chlorophyll content and nitrogen content of shoot and root in soybean. The nodules number continuously

content of shoot and root in soybean. The nodules number continuously decreases from 5% onwards upto 100% in soybean. Jahan (1993) found that plant growth,leaf chlorophyll, nitrogen and phosphorus contents, seed protein, number of VAM spore and root nodulation of chickpea and mungbean were increased at low levels of fly ash (10 to 25%) and decreased from 50 to 100% levels of fly ash, but root colonization by VAM fungus decreased from 10 to 100% levels of fly ash.

Mycorrhizal Fungi

Mycorrhiza refers to symbiotic relationship between fungi and plant (Frank, 1885). Plant roots are normally infected by mycorrhizal fungi, which are non-pathogenic. Growth of the higher plants is frequently enhanced by the development of mycorrhizal roots (Smith, 1980). Mycorrhizal colonization is responsible for increased growth and nutrient uptake in natural ecosystem. Mycorrhizal fungi were first divided into ectotrophic and endotrophic mycorrhizae (Harley, 1969). They were later divided into ectomycorrhizae, endomycorrhizae and ectendoycorrhizae, the last being described as an intermediate form. Endomycorrhizae are subdivided into vesicular-arbuscular, erricoid and orchid mycorrhizae. The latter two occur in association with the plants of the families Ericaceae and Orchidaceae, respectively (Harley and Smith, 1983).

Vesicular-arbuscular mycorrhizal fungi (VAM)

VAM fungi are characterized by the presence of two specialized structures, vesicles and arbuscules. Arbuscules are formed by the internal mycelium intracellularly as hyphae penetrate mechanically and enzymatically into cortical cells in the form of highly ramified aborescences within a few days of infection (Kinden and Brown, 1975c, Rich and Bird, 1974). Bifurcated arbuscule hyphae are enveloped by a host derived encasement layer (Cox and Sanders, 1974 ; Scannerini and Bonfante-Fasolo,1979) and continuously invaginate host plasmalemma and form a wide contact between the two symbionts but are short lived and can be digested within few days after formation by host (Cox and Sanders, 1974; Kinden and Brown, 1975a,b; Dexheimer *et al.*, 1979). Vesicles are also produced by internal mycelium intercellularly and are known to be a storage structures. These fungi taxonomiclly belong to the family Endogonaceae of the order Endogonales in the class Zygomycetes. Gerdemann and Trappe

(1974, 1975) recognized five genera i.e. *Glomus, Gigaspora, Endogone, Acaulospora, Sclerocystis* which form symbitoic associations with plant roots. According to Morton and Benny (1990) they are grouped into separate order Glomales in Zygomycetes.Trappe (1982) gave a synoptic key to the genera of Endogonaceae and described nine genera including *Complexipes* and *Modicella*.Schenck and Perez (1987) reported 120 species of soil fungi forming VA mycorrhizae. These genera are distinguished from each other on the basis of the manner of spore formation by them. Several characteristics which are considered to be important are the presence or absence of sporocarps, spore colour and cell wall thickenss, number of cell wall layers, ornamentation, as well as shape and number of hyphal attachment on the spore. *Glomus* forms chlamydospores at terminal position on a single undifferentiated hypha. Some of its species form sporocarps while others are incapable of doing so. *Gigaspora* is a non-sporocarpic, forming azygospores on a large suspensor. *Acaulospora* is also a non-sporocarpic member producing azygospore at a lateral position singly on a hypha which terminates in a large vesicle. In *Sclerocystis*, the chlamydospores are arranged tightly in a single layer around a central plexus of sterile hyphae.

VAM fungi are woridwide in distribution. They are found in soil in form of chlamydospores, zygospores and azygospores. VAM fungi have been isolated from the soils of a variety of habitats. Spores of *Acaulospora laveis* also called "honey coloured sessile" have been reported in Australia, Brazil, England, New Zealand, Pakistan, Scotland, South Africa and the U.S.A. (Gerdemann and Trappe, 1974; Mosse and Bowen, 1968). Redhead (1977) found VAM in all 15 exotic and 44 out of 51 indigenous plant species in a lowland tropical rain forest in Nigeria. St. John (1980) reported an abundance of VAM in species with magnoleoid root systems in a Brazilian rain forest. Woody plants from the 'cerrado' regions of Brazil were also reported to have endomycorrhiza (Thomazini, 1974).

VAM is also abundant in many temperate grasslands. Crush (1973) found both VAM infections and resting spores to be widespread in the native tussock hill grasslands of New Zealand. Read *et al.* (1976) found that plant species of family Graminae were heavily infected with VAM in the semi-natural hill grassland in England.Much VAM infection but few spores were reported in acid hill grasslands in northern England, mid-Wales , Western United States and Canada (Sparling

and Tinker, 1978; Hayman and Mosse, 1972; Molina *et al.*, 1978).Species of *Gigaspora* were abundant in hot climates of Australian heaths, sand dunes (Sward *et al.* 1978, Koske, 1975) and *Glomus* and *Acaulospora* species were abundant in New Zealand (Crush, 1973, 1975). Out of 40 plant species belonging to 15 families, 88% were found to be heavily infected with VAM first recorded on natural vegetation systems of Iraq (Franics and Merivani, 1988). O'Dell and Trappe (1992) reported VAM infection of lupin and some other legumes in North western U.S.A. VAM fungi are also reported from diverse habitats in India (Godse *et al.*, 1976; Bagyaraj *et al.*, 1979b; Parvathi *et al.*, 1984; Kheri *et al.*, 1987;Chandra and Chatterjee, 1989;Kheri and Chandra, 1990).

VAM mycorrhizal associations occur widely throughout the plant kingdom (Gerdemann, 1968). They have been reported to be present in Bryophytes, Pteridophytes, Gymnosperms and Angiosperms. They are absent only from a few plant families. He listed 14 families which are believed to have little or no mycorrhizae including the Cruciferae (Brassicaceae), Chenopodiaceae, Caryophyllaceae, Polygonaceae, Juncaceae and Cyperaceae. High incidence of infection is found in tropical and temperate forest trees (Baylis, 1961; Alwis and Abeybayke, 1980; Thapar and Khan, 1985).

VA mycorrhizal associations in number of plant species have been given by several workers. Janse (1897) and Gallaud (1905) were the first who described the associations of these fungi with roots that formed the endotrophic mycorrhizae. After them several other workers have described the VA mycorrhizal association in a number of plant species such as soybean, clover, onion, ryegrass, bean, sorghum, poinsetta, banana, pine apple etc. (Nicolson 1959; Schenck, and Hinson, 1973; Bagyaraj *et al.*, 1979a; Ramesh, 1984, Iyer Rohini *et al.*, 1988; Mosse *et al.*, 1982; Miranda *et al.*, 1989; Jaizmevege and Azcon, 1991; Clapperton and Reid, 1992).

Root morphology show a little or no obvious change due to development of mycorrhizae. In some plants yellow pigmentation accompanies root colonization and the endodermis may become thickened. Development of endomycorrhizae results in loss of root hairs, but no external fungal mantle forms except for the relatively sparse external hyphae that occur at the rhizoplane and extended out into the soil (Carling and Brown, 1982). Graham *et al.* (1981) proposed that under low phosphorus nutrition VAM increased root membrane permeability and

exudation compared to non-mycorrhizal plants.

The physical and chemical effects of the fungal symbiont hyphae in surrounding soil, result in a very different potential in the rhizosphere 'Mycorrhizosphere'.This term was suggested by Rambelli (1973) to be used to describe the soil surrounding and influenced by mycorrhizae. Extramatrical hyphae that extend out some distance from the host tissue into the soil have profound effect on the soil microflora (Graham *et al.*, 1982; Rhoads and Gerdemann, 1978). Extramatrical hyphae of VAM fungi exude substances that cause soil and organic fractions to aggregate (Sutton and Sheppard, 1976). Forster and Nicolson (1981) observed that microorganisms flourish in the aggregates and fungi, bacteria, actinomycetes and algae (including cyanobacteria) have been isolated from them.

Nutritional interactions

Availability of essential mineral elements, commonly N, P and K in poor soils is a major limiting factor for plant growth. Addition of these elements as fertilizers to most natural soils results in improved plant growth. Various experiments conducted on the uptake of phosphate show that its uptake is enhanced by mycorrhizae. Phosphate is a nutrient required in relatively large amounts by plants. Much of the phosphate in the soil is in insoluble form not readily available to plants and it is frequently in low concentration (micromolar or less) in the soil solution. Inorganic phosphate tends to become immobile because it binds to soil colloids, or is fixed as aluminium and iron phosphates. Consequently, roots may take up phosphate from the soil more quickly than phosphate diffuses to them, resulting in the formation of a "zone of depletion" around them (Nyke and Tinker, 1977). Slow rate of diffusion of phosphate into the zone of depletion causes limited uptake by the root zone. The external mycelium of a mycorrhizal fungus grow through this zone to the soil beyond, therefore, its uptake is not limited, especially if hyphae continue to grow and explore new volumes of soil. Mosse (1976) observed the effect of VAM in improving phosphate supply of plants results from the absorbing capacity of extensive network of external hyphae associated with the infected roots.

More recently various workers have shown that the hyphae of VA mycorrhizae also transport ^{32}P from the soil to the host. Six mycorrhizal fungi

were tested as inoculant for pearl millet grown in pots maintained in a greenhouse. VAM fungi varied in their ability to stimulate plant growth and phosphorus uptake. Inoculation with *Gigaspora margarita, G. calospora* and *Glomus fasciculatum* increased shoot dry matter 1-3 fold over uninoculated control. In an other pot trial, inoculation with *G. calospora* and *G. fasciculatum* resulted in dry matter and phosphorus uptake equivalent to that produced by adding phosphorus at 8 kg/ha (Krishna and Dart, 1984). The efficiency of utilization of various forms of P mineral by mycorrhizal plants depends on the species of mycorrhizal fungi present and on the soil type. When soybean plants were inoculated with *Glomus fragilis* and *Glomus fasciculatum* in four different types of soil, yield of soybeans and phosphorus uptake was increased in *G. fasciculatum* inoculated plats than *G. fragilis* inoculated plants (Young *et al.*, 1986). Mc Gonigle and Fitter (1988) showed that the rate of P uptake per unit root length in *Trifolium repens* was proportional to the extent of mycorrhizal infection. Studies on phosphorus transport by hyphae of the 3 VAM fungi i.e., *Acaulospora laevis, Glomus* sp. and *Scutellospora calospora* associated with *Trifolium subterraneum* showed that after 37 days *S. calospora* transported much less ^{32}P to plants, *Glomus* sp. to shorter than 1 cm, *Acaulospora laevis* transported over a longer distance than the two fungi (Jakobsen *et al.*, 1992). Pearson and Jakobsen (1993) found that hyphae of *G. caledonicum* were most rapid in 32 P uptake and transfer in cucumbers among 3 VAM fungi i.e., *Glomus* sp., *Scuttellospora calospora* and *Glomus caledonicum.*

In the studies on uptake of ^{15}N from the soil, Ames *et al.* (1983) found that different sources of nitrogen along hyphae of VAM fungi were similar to those of phosporus. Baath and Spokes (1989) studied the effect of different levels of phosphorus and nitrogen on mycorrhizal growth response and infection by *Acaulospora schoenoprasum* and *Glomus caledonicum.* Both the level of soil phosphorus and level of nitrogen added affected the mycorrhizal growth response, which was greatest at intermediate levels of P and N. Root colonization by VAM fungi i.e., *Glomus etunicatum, Giagospora margarita* was found to be often suppressed by phosphorus additions, however, nitrogen addition both stimulated and suppressed root colonization of both VAM fungi in *Allium cepa* (Sylvia and Neal, 1990). Smith *et al.* (1985) and Azcon *et al.* (1992) found that mycorrhizal infection increased the glutamine synthetase activity in clover, onion and lettuce, and they suggested that this enzyme, which has a high affinity for ammonia, may

be important in the uptake of ammonia by soil hyphae.

Vesicular-arbuscular mycorrhizal association can also play an important role in obtaining increased yields by Zn supply to plants from soil. Barber (1962) gave three processes for mobilization of nutrients from soil to be surface of plant roots, namely mass flow, root interaction and diffusion. Among these, diffusion is considered to be the most important since it is often the rate limiting process in mobilization of Zn to plant roots (Wilkinson *et al.*, 1968). In greenhouse experiments with moongbean in soils fertilized with graded levels of Zn (0, 25 and 5 mg/kg soil), treatement with *Glomus macrocarpum* increased Zn mobilization to plant roots (Sharma *et al.*, 1991). Rhodes and Gerdemann (1975) have shown that mycorrhizal plants are able to extract more nutrients from the soil through their extra-matricular mycelium which in certain cases extends upto 8 cm beyond the root surface and thus facilitates easier absorption in a nutritionally poor zone. Mycorrhizal exudates are capable of forming soluble zinc complexes in the rhizosphere, due to which fixation of Zn in soil is reduced, diffusion is improved and the availability of zinc to plants increased (Cakmak and Marschner, 1988).

Root Nodule Bacteria

Symbiotic nitrogen fixation by root nodule bacteria in leguminous crops is a very significant natural biological process. Elkan (1984) reorganised species of root nodule bacteria and created a new genus *Bradyrhizobium.* According to him the genus *Rhizobium* consists of three reorganised species, *R. leguminosarum*, containing three biovars (biovar *trifolii*, biovar *phaseoli* and biovar *viceae*), *R. meliloti* and *R. loti.* The reorganisation combined into one, the former species of *R. leguminosarum, R. trifolii* and *R. phaseoli.* The fast growing members of the cowpea rhizobia and the former species *R. lupinus* were included in species *R. loti.* They are fast growing, subpolar flagellated strains from *Lotus and Lupinus*, with strong affinity for *L. corniculatus, L.densiflores* and *Anthyllis vulneraria* (but also nodulates *Ornithopus sativum*). It also includes the fast growing strains nodulating *Cicer, Sesbania, Leucana, Mimosa* and *Lablab.* The new genus, *Bradyrhizobium*, has one species, *B. japonicum*, which consists of the former species *R. japonicum* plus the slow growing members of the cowpea rhizobia. They are slow growing, polar or subpolar flagellated strains nodulating soybean, *Lotus uliginosus, L. pendutulatus* and *Vigna.* It also includes slow growing strains nodulating *Cicer, Sesbania, Leucana, Mimosa, Lablab* and *Acacia*.

Root nodule forming bacteria live freely in soil and in the root region of both leguminous and non- leguminous plants. They develop association with the roots of leguminous plants and form nodules on them. As root nodules become older after a period of nitrogen fixation, decay of tissue occurs, liberating a motile forms of *Rhizobium* into the soil, thus providing a source of inoculum for the succeedding crop of a given species of the legume (Subba Rao, 1972, 1975). The bacteria after penetrating through root hair, form the nodule in the upper cortical regions. The core of mature nodule constitutes the bacterioid zone surrounded by several layers of cortical cells; they show a direct positive realationship with nitrogen fixation. The effective nodules are generally large and pink in colour due to leg-haemoglobin (Bergersen and Briggs, 1958).

Nitrogen fixation is directly and indirectly influenced by the effects of soil acidity on bacteria and on the host. *Rhizobium* species and strains vary in their tolerance to soil pH. Among tropical and temperate legumes, both tolerant and intolerant species are found and they have different responses to lime (Munns, 1976; Munns *et al.*, 1977).

According to Freire (1976) the effect of soil acidity and pH are related factors on soybean nodulation and N_2-fixation. Calcium is required in adequate amounts for development of well defined nodulation, growth and activity of *Rhizobium.* Soil acidity and availability of nuitrients (such as macro- and micro - nutrients as Ca, P, Mn, Al, B, Mo and Cu) uptake to plants are correlated to each other. Lime applicatons correct Al and Mn and the availability of many other elements in soil (Andrew, 1976).

Rhizobium phaseoli can grow at limiting pH 4.0 to 4.4 in liquid media. Paulino *et al.* (1987) observed a decrease in the number of nodule formation and acetyelne reduction of pea plants infected with *R. leguminosarum* at low pH 5.2. In tropical and subtropical areas soil temperature was a major limiting factor for beans (Graham and Halliday, 1976).

Kluson *et al.* (1986) reported that temperature affected nodule formation by *R. japonicum* on soybean. Its growth both in broth and soil were also affected by temperature. The competitiveness of all inoculum was increased, when temperature was raised from 20 to 35°C. Vegetative growth of soybeans and nodulation were minimum at 20°C and optimum at 30°C.

Keyser and Munns (1979) studied the direct effect of Al and Mn toxicities on rhizobia. They found that when toxic levels of Al and/or Mn are persent in the soil, the limiting amount of P fertilizer was required for promoting adequate nodulation. Andrews (1976) observed that in acid soils Mn toxicity and Ca deficiency was less important factor than Al toxicity and acidity, which was increased in pasture legumes. Geopfert (1971) reported that grain yield of soybean, P availability in the soil and nodule weight of soybeans had a close relationship.

Becana and Janet (1989) showed a significant reduction in the total content of Lb in *Glycine max* ,*Pisum sativum*, *Trifolium repens*, *Vigna radiata*, and *Vigna anguiculata* except in *Lupinus luteus*, when all the nodules of each host were treated by $NO^{-}3$.

Rhizobia are also affected by application of fungicides /insecticides in soil. Five fungicides (Dithane M-45, thiram, blitox, captan ,emision) and four insecticides (thionel, malathion, aldrin and BHC 50) at different concentrations, were tested for their effect on different *Rhizobium* sp. Most of the chemicals were non-inhibitory to *Rhizobium* at lower concentrations. At higher concentrations, the chemicals became inhinbitory but the response of vaarious *Rhizobium* sp. to these chemicals was different (Poi and Ghosh, 1986).

Interaction between VAM fungi and root nodule bacteria

Nodulation of various legume species has been shown to be responsive to the inoculation with mycorrhizal fungi. Interaction between VAM fungi and root nodule bacteria was found to be synergistically beneficial for plants. Bagyaraj and Menge (1978) observed an increased mycorrhizal colonization on dual inoculations with VAM fungi and rhizobacteria. The microbial interactions in the mycorrhizosphere of VA mycorrhizae have been reviewed by Barea and Azcon-Aguilar (1982) and Bagyaraj (1984). Increase in rhizosphere populations of bacteria and actinomycetes has been reported by Bagyaraj and Menge (1978), when plants were inoculated with *Azotobacter* or VAM, inoculated singly or in combination. The increase was highest from the combined inoculation of both microorganims. Meyer and Lindermann (1986) observed no quantitative differences in total bacteria in the rhizosphere soil from mycorrhizal plants, but significant qualitative shifts were found, on comparing the rhizoplane and rhizosphere soils with regard to the selection of qualitatively different functional

groups of bacteria from the naturally occurring microflora.

Mycorrhizal inoculation enhances nodulation in legumes (Crush, 1974; Smith and Daft, 1977; Carling *et al.*, 1978). Phosphorus level influences not only mycorrhizal infection frequency but also the process of nodulation in leguminous species since the legumes are poor competitor for soil phosphates. Ho and Trappe (1973) detected the capability of *Glomus macrocarpum* and *G. mosseae* to reduce nitrate to nitrite. Mycorrhizal nodulated soybean plants inoculated with *Glomus fasciculatus* and *Rhizobium* exhibited higher levels of nitrogenase and nitrate reductase as compared to non-mycorrhizal plants (Carling *et al.*, 1978; Bagyaraj *et al.*, 1979a). Inoculation of *Medicago sativa* with 3 isolates of VAM fungi belonging to the species *Glomus mosseae, G. fasciculatum* and *G. caledonicum* in dual combination with six strains of *R. meliloti* resulted in increased plant growth, N and P nutrition as compared to single inoculation. This effectiveness was in order: *G. fasciculatum ,G. mosseae, G. caledoncium* (Azcon *et al.*, 1991). Dual inoclation with VAM and *Rhizobium* resulted in better utilization of phosphorus, increased nitrogenase activity in nodules and increased nitrogen fixing ability in host plants (Tilak, 1991).

Effects of inoculation of *Hedysarum boreale* with mycorrhizal fungi and *Rhizobium* were studied in the field. After 3 years, plants receiving both had greater total above ground biomass and leaflet biomass, more leaves, higher above ground nitrogen and phosphorus contents and greater survival than plants which received single or no inoculum (Alan, 1988). Badar El-Din and Mowad (1988) observed a combined effect of VAM and *Rhizobium* on lentil, fababean and soybean in soils with low indigenous VAM spores. The inoculation of the plant with VAM fungi increased the level of mycorrhizal root infection of the three legume. The inoculation with *Rhizobium* had no significant effect on percent VAM infection, but VAM inoculation increased nodulation of the three legumes. When plants of chickpea (*Cicer arietinum*) were dual inoculated with VAM fungi and *Rhizobium* showed a maximum utilization of P from mono- and dicalcium phosphate than plants receiving VAM or *Rhizobium* inoculum alone. Uninoculated plants showed minimum uptake and translocation of P from labelled phosphatic fertilizers(Chaturvedi and Sharma, 1988).Adholeya *et al.* (1988a) observed a greater yield, dry weight and N-uptake in plants of *Vigna radiata* inoculated with both mycorrhizae (*Glomus caledonicum*) and *Rhizobium* (Strain No. KM-1) than

mycorrhizal or rhizobial alone and uninoculated plants. According to Adholeya *et al.* (1988b) maximum infection rate, nodule number, nodule dry weight, nitrogenase activity occurred in *V. radiata* plants which were inoculated both with mycorrhizal fungus and *Rhizobium* in field. The effect of *Glomus macrocarpum* was more prevalent in early stages of *Rhizobium* interaction and in soil with low P concentration and in controlled conditions. VAM fungi showed a tendency of increasing nitrogenase activity when applied with *Rhizobium.* VAM fungi *Acaulospora scrobiculata*, *Glomus mosseae, G. fasciculatum, G. intraradices, G. claroideum, Gigaspora margarita* and *Scutellospora spinosa* also increased growth of legumes, citrus, maize in greenhouse and in field. (Vasuvat, 1991).

Dual inoculated plants of *Medicago sativa* and *Trifolium alexandrinum* showed an increased dry weight (73% and 22% respectively) as compared to plants inoculated singly (Patterson *et al.*, 1990). Murakami-Mizukami *et al.* (1991) studied the changes in the hormonal balance of the plants having VAM fungi with nodules. Indole acetic acid (IAA) and abscissic acid (ABA) contents of soybean plants with *Bradyrhizobium japonicum* nodules and associated with VAM fungus *Glomus etunicatum* were determined. The IAA content in nodules, ABA contents in roots, shoots and nodules of mycorrhizal plants was higher than non-mycorrhizal plants. Dual inoculated plants with *G. fasciculatum* and *B. japonicum* of mungbean and chickpea showed an increase in plant growth, yield, nitrogen and phosphorus contents of root and shoot, chlorophyll content of leaves, protein of seeds than single inoculated by either one of them or uninoculated plants (Jahan, 1993).

Interactions between VAM fungi and other microorganisms

Soil-borne pathogens causing particularly wilts and root-rots are influenced by microbial activity in rhizosphere of plants. Vesicular-arbuscular mycorrhizal colonization promote or inhibit the rhizosphere microorganism (Schonbeck, 1979).

Mycorrhizal root systems are less susceptible to the attack of soil pathogens than non-mycorrhizal systems. Becker and Gerdeman (1977) reported that roots of onion were less susceptible to *Pyrenophora terrestris* causal agent for pink root disease. Mycorrhizal root segements exhibited more resistance than non-mycorrhizal segments of the root, suggesting that quantum of mycorrhizal colonization is directly proportional to disease resistance.

Jalali and Thareja (1981) demonstrated that mycorrhizal inoculations resulted in significant reduction in the host infection by *Fusarium* and *Rhizoctonia*. Drastic growth suppression of *Fusarium oxysporum* f. sp. *ciceri* in chickpea was observed when subjected to soil inoculation with *Glomus* sp. Similar response was observed on *Rhizoctonia solani* when seed-pelleting with sporocarps of the mycorrhizal endophyte was done (Jalali, 1983).

Shoot and root weights and percentage of healthy roots of mycorrhizal (*Glomus fasciculatus*) sweet orange inoculated with *Phytophthora parasitica* were greater than those of non- mycorrhizal seedlings (Davis, 1981). Growth of sour orange seedlings was increased by mycorrhizal fungi like *Glomus intraradices, G. fasciculatum, Gigaspora heterogona, Sclerocystis coremiodies* but decreased by *Phytophthora parasitica* and *Thielaviopsis basicola* (Davis *et al.*, 1986). Studies of Jalali *et al.* (1990) on the interaction between VAM fungus *Glomus mosseae and Macrophomina phaseolina* in mungbean revealed that disease incidence was reduced from 77.9% in pathogen inoculated to 13.3% in mycorrhizal plus pathogen inoculated plants. Dual inoculation with mycorrhizal endophyte and the pathogen also resulted in increased total dry matter production, nitrogen, phosphorus and potassium contents of such plants as compared to pathogen inoculated plants. The effect of inoculation with *Erwinia carotovora* pv. *carotovora* on tomatoes with or without VA mycorrhizal infection by *Glomus mosseae* was studied by Garcia- Gamdo and Ocampo (1988) in greenhouse .The growth of non- mycorrhizal tomato plant decreased.

Mycorrhizal fungi inhibit the development of plant parasitic nematodes. Oliveria and Zambolin (1986) observed an increase in dry weight of the shoot, pod yields, nutrient uptake and decreased in egg production by the nematode in plants inoculated with *Glomus etunicatum* and *Meloidogyne javanica* in comparison to the plant inoculated with *M. javanica* alone. The stunt nematode *Tylenchorhynchus vulgaris* showed an interaction with a VAM fungus on *Trifolium alexandrinum* in which the VAM fungus offset the nematode damage and increased the plant growth and phosphorus content of the plants in greenhouse experiment (Hasan and Jain, 1987). When rough lemon seedlings were grown in mycorrhizal infested (*Glomus intraradices*) or phosphorus amended soil (25 and 300 mg/kg) in greenhouse experiment and inoculated with citrus burrowing nematode, *Radopholous citrophilus*, after 6 months mycorrhizal

and non-mycorrhizal high P plants had larger shoot and root weight, lower nematode population as compared to non- mycorrhizal, low P plant (Smith and Kaplan, 1988). Jain and Sethi (1989) studied the effect of early establishment of *G. fasciculatum* or *G. epigaeus* on the penetration and development of *Heterodera cajani* in cowpea (*Vigna unguiculata)* in pots. Over 60% colonization of the root system by VAM fungi considerably hampered root invasion by the nematode. VAM fungus in a greenhouse study on cotton did not affect plant growth or population density of *M. incognita*, determined 30 days after simultaneous inoculation at planting, but caused significant increase in plant growth and reduction in *M.incognita* population density and reproduction after 50 days of inoculation (Saleh and Sikora,1989). The effects of mycophagous nematodes *Aphelencoides composticola* on mycorrhizal *Glomus clarum* and non-mycorrhizal red clover (*Trifolium pratense)* plants grown in soil with or without *Agaricus bisporus* were recorded by Giannakis and Sanders (1990). All pots containing *A. bisporus* had higher nematode numbers, while *G.clarum* inoculated pot had lower nematode number.

Effect of inoculation with *Glomus fasciculatus, Beijerinckia mobilis* and *Aspergillus niger* singly and in combination, on growth and nitrogen, phosphorus contents of onion was studied by Manjunath *et al.* (1981). Inoculation with *G. fasciculatus or B. mobilis* increased the dry weight and nitrogen content of the plants. *A. niger* inoculation had no significant influence on plant growth. *B. mobilis*, inoculation stimulated the sporulation by *G. fasciculatus.* Synergistic beneficial effect occurred due to inoculation with all the 3 organisms.

Effect of gaseous pollutants on mycorrhizae

Since most plants are adversely affected by air pollution, mycorrhizae because of their symbiotic association in he plant roots are liable to be affected by the pollution. There are various reports on the effect of gaseous pollutants on mycorrhizae. Some of them are on ectomycorrhizae. The direct effects of pollutants on mycorrhizal hyphae were demonstrated by a 65-85% reduction in respiration of *Pisolithus tinctorius and Thelephora terrestris* mycelium in pure culture after 1 h exposure to 50 and 500 ppb O_3 and SO_2 (Garrett *et al.*, 1982). When four- week-old paper birch seedlings inoculated or non-inoculated with the ectomycorhizal fungus *Pisolithus tinctorius* and grown in steamed or non-steamed soil, were exposed to O_3 for 7 h/day or 5 day/week for 12 weeks at 0.06 and 0.08

ppm concentration and simulated acid rain was applied 10 min/day on 2 days/week interactions between O_3 and simulated acid rain, soil regime and mycorrhizal treatment had an adverse effect than individual treatment (Keane and Manning, 1988). Shafer *et al.* (1985) showed that mycorrhizal formation on *Pinus taeda* declined with reduced pH in the presence of SO_4 and NO_3 but increased at the lowest pH of 2.4. Synergistic interactions between Al concentration and O_3 in reducing mycorrhizal development of *Pinus rigida* by *Pisolithus tinctorius* have been shown to occur (Mc Quattie and Schier, 1987).

Effects of air pollutants reported include inhibition of sporulation and endomycorrhizal associations which increase metal uptake (Bewer and Heagle, 1983; Killham and Firestone, 1983). Variable effects on infection by ectomycorrhizal infection depends on the level of acid deposition (Shafer *et al.*, 1985). Significant decrease in the amount of VAM infection in the roots of *Phleum pratense* exposed to SO_2 occurred. Low concentrations (0.05 to 0.07 ul/l) of SO_2 affected the ability of endomycorrhizal fungi to colonize and also to proliferate within roots (Clapperton *et al.*, 1992). Ozone suppressed infection of roots by an endomycorrhizal fungus (Mc Cool *et al.*, 1982; Mc Cool and Menge, 1984) and suppressed sporulation (Bewer and Heagle,1983). Effects of air pollution on ectomycorrhizal relations have been varied (Garrett *et al.*, 1982; Mahoney *et al.*, 1985; Reich *et al.*, 1985,1986; Keane and Manning, 1988; Meir *et al.*, 1990).

Exposure of mycorrhizal *Festuca arundinacea* to 0.1 ppm $O_3$for 3 months reduced root weights and intensity of mycorrhiza formation as compared to plants in filtered air. This response was attributed to reduced photosynthesis by O_3 exposed plants (Ho and Trappe, 1984). The effect of acid rain and ozone on soybean plant with endomycorrhizal fungus *Glomus macrocarpus* was observed by Fiecht (1981). The simulated acid of pH 3.2 or 2.8 did not affect percentage root colonization by *G. macrocarpus* but ozone reduced spore production of *G. macrocarpus* without reducing colonization. The total number of chlamydospores/unit dry weight of root was reduced by 0.08 ppm O_3 (Fiecht, 1981).

Jagpal *et al.* (1988a) determmined the effect of automobile pollution on VAM spore population of soil from different traffic intersection of Delhi for 4 to 6 months. The changes in spore population were determined both for soil from surface and 6 inches depth. The traffic density and lead concentration were negatively

correlated with spore number. The air pollutants SO_2 and O_3 at 0.1 ppm and 0.2 ppm reduced root colonization and number of spores of *G. fasciculatum* in chickpea and mungbean plants (Jahan, 1993).

Effect of air pollution on nodules and nodulation

Several reports show impacts of air pollution on nodules and nodulation of the host plant by root nodule bacteria. The major air pollutants implicated are SO_2, O_3, acid rain and fly ash.In greenhouse or conrtrolled environment acute O_3 exposures to leguminous plant, reduced the *Rhizobium* nodulation, nitrogen fixation and/or leghaemoglobin content (Blum and Heck, 1980; Blum and Tingey, 1977). Reinert and Weber (1980) pointed out that exposures of soybean plants to 0.25 ppm O_3 for 4 h/day, 3 days/week, for 11 weeks in greenhouse, reduced the number of *Rhizobium* nodules/plant and nodule weight/plant by 46 and 41% respectively.At pH 3.2 sulphuric acid reduced the *Rhizobium* nodulation of kidney beans grown in a grenhouse or field plots and of soybeans grown in greenhouse (Shriner, 1974; Shriner, *et al.*, 1981; Waldron, 1978). Nodulation in soybeans was not reduced by acid rain of pH 3.5 grown in the field plots (Mc Gruit, 1976). Waldron (1978) separated the effects of H^+ and SO^-4 ions on nodulation by rain or soil drench application of sulphuric acid (pH 3.2) or sodium sulphate (pH 5.7). He observed that SO^-4 ions caused a slight reduction in nodulation when applied as `rain' while H^+ions caused thae major inhibition in nodulation. The inhibiton of nodulation also occurred due to substance which was leached by rain from the foilage (Shriner, 1974; Waldron, 1978). Reduction in the number and dry weight of the bacterial nodules occurred, on exposure of soybean plants to O_3 (Reinert and Weber, 1980). Klarrer *et al.* (1984) showed that nodule number was significantly reduced, while there was no effect on individual nodule size, when soybean plants were exposed to SO_2 and NO_2 alone or in mixture. This reduction in nodule number was due to reduction in N-fixation.

Acidity of the medium was found to be correlated with the growth inhibition of chickpea and lentil strains of *Rhizobium* by Singh (1989). At pH 6, growth of chickpea strain were significantly unaffected. The chickpea strain of *Rhizobium* was apparently more sensitive to acidity than lentil strain. At pH 3.2 total growth inhibition of chickpea strain occurred while lentil strain growth was inhibited at pH 2.5. Total number of nodules and functional nodules decreased significantly at both the pH of rains in chickpea and lentil strain. The growth of chickpea and lentil

strain of *Rhizobium* was suppressed, when exposed to SO_2at concentration 0.1 ppm and 0.2 ppm and O_3 at same concentration alone or in combination. Inhibitory effects were more at the higher concentrations. Chickpea strain had greater sensitivity to SO_2 exposures and less sensitivity to O_3 exposure than lentil strain. There was greater reduction in the growth of chickpea strain than lentil strain exposed to a mixture of SO_2 and O_3 at both concentrations. Root nodulation (number of nodules/root system) was also reduced (Singh, 1989). The number of nodules and nodule dry weight of soybean were reduced at 0.1 ppm and 0.2 ppm concentrations of SO_2 and O_3, respectively (Singh, 1993).

In artificially amended soil with fly ash at different levels i.e. from 10 to 100% showed a stepwise decrease in the nodule formation of *Rhizobium,* with an increase in levels of fly ash of soil and was completely suppressed at 70 and 80% levels of fly ash in chickpea and lentil strains, respectively (Singh, 1989). Suppression of root nodulation in fly ash amended soil occurred in both chickpea and lentil . The nodule number and dry weight of nodules continuously decreased with an increase in levels of fly ash in soybean (Singh, 1993). The nodule number and dry weight of nodule of mungbean and chickpea was increased from 10 to 25% level of fly ash and decreased with further increase in fly ash level (Jahan, 1993;Abbas, 1994).

It is evident that root symbionts like root nodule bacteria and VAM fungi develop symbiotic relationship with legumes and provide nutritional benefits to the crop plants. This favourable effect of the symbionts is likely to be influenced by any stress on the host which may affect the both the components of the symbiotic system, the host plant as well as root symbionts. Air pollution which is new stress factor of modern times is expected to influence the system in the same way. This aspect,however,has gained very few studies. Information available through such studies indicate towards such an important impact on the symbiotic system, where both the host and the symbionts suffer.Since the system of symbiotic relationship of plants and root symbionts is of great agricultural significance,it is imperative to investigate its various aspects,so that the adverse impact of air pollution on plants and associated microorganisms can be fully realized. The present study carried out under controlled conditions is an effort in this direction.

MATERIALS AND METHODS

The experiements were conducted by using sulphur dioxide (SO_2) and ozone (O_3) as gaseous air pollutants for exposure of the experimental plants and fly ash as a particulate air pollutant for incorporation in soil. The effects were determined on plant growth and yield of black gram (*Vigna mungo* (L) Hepper), an important leguminous crop in India and on their root nodulation by root nodule bacterium, *Rhizobium* sp. nodulating on black gram (*Vigna mungo* L.) and root colonization by VAM fungus, *Glomus caledonicum* (Nicol. and Gerd). Trappe and Gerdemann. Preliminary survey of fields grown with black gram in and around Aligarh showed the *Glomus caledonicum* was common VAM fungus infecting the roots of the plants. Isolation of spores from soil samples collected from the same field yielded higher number of *Glomus caledonicum* spores than other VAM fungi. Therefore, this VAM fungus was selected for the experimental work in this study. The details of materials used and methods employed in the study are given below.

VAM FUNGUS

Starter culture of VAM fungus (inoculum production)

(a) Collection of soil samples in the study

Glomus caledonicum was used as VAM fungus. In order to collect spores of *G. caledonicum*, fifty soil samples were collected from the crop fields in Aligarh and adjoining areas grown with black gram (*Vigna mungo*). Samples were collected with the help of soil auger upto depth of 15 cm underneath the plants.

(b) Isolation of spores of VAM fungi from the soil samples

Spores of VAM fungi present in the soil samples were isolated by wet sieving and decanting method (Gerdemann and Nicolson, 1963). A sample of 100 g dry soil was mixed in water (1000 ml) and the heavier particles were allowed to settle for few seconds. The liquid was poured

liquid passed through coarse sieve was collected and again passed through a series of sieves of varied size i.e. 80, 150, 250 and 300 mesh. Spore obtained on sieves were collected with water in separate beakers. Repeated washings with Ringer's solution (NaCl 6g l^{-1}, KCl 0.1g $l^{-1}$and $CaCl_2$ 0.1 g l^{-1} in D.W., pH 7.4) isolated and recovered most of spores from soil detritus. Spores of VAM fungi were identified under a dissecting microscope (15X) with the help of the synoptic keys (Trappe, 1982) and the spores of *G. caledonicum* were separated by picking and used for pot culture. Spores were separated with a microspatula and picked up by a pasteur pipette fitted with a rubber bulb. These were surface sterilized for 2 minutes in a solution containing chloroamine T 20g/l, streptomycin 300 mg/l and tween 80 a trace amount/l in distilled water.

(c) Culture

Culture of *G. caledonicum* was raised on black gram (*Vigna mungo*) plants grown in pots under glasshouse conditions. Seeds of black gram cv. Type 9 Pantnagar were surface sterilized with 0.1% solution of $HgCl_2$. The surface sterilized seeds were dressed with soil-based culture of *Rhizobium* sp. and planted (5 seeds per pot) in 50 clay pots of 9 cm diam. containing sterilized field soil (66% sand, 24% silt, 8% clay ,OM 2%, pH 7.5). Fifty spores of *G. caledonicum* per pot were layered at 6 and 2 cm. in each clay pot. After emergence, seedlings were thinned and one seedling was maintained in each pot. After 125 days, the plants were uprooted and the spores were isolated by wet sieving and decanting method from the pot soil. Roots were examined for the VAM colonization.

Inoculation of VAM fungus

Spores of *G. caledonicum* were used as inoculum for the experiments. The spores were obtained from the pot culture of *G. caledonicum* raised on black gram, as described above. Spores were applied at the rate of 1000 spores/pot as thin layers 6 and 2 cm below the surface of the soil before sowing the seeds in experimental pots.

ROOT NODULE BACTERIUM

Isolation of root nodule bacterium

Rhizobium sp.was isolated from root nodules present on roots of black gram plants collected from fields. After washing the root system of the plants in running water, a well formed healthy pinkish nodule on the tap root was carefully cut out with a portion of root attached to the nodule. The nodule was suface sterilized for 5 min. in 0.1% mercuric chloride and repeatedly washed with sterilized distilled water to remove the chemical. The nodule was then washed in 70% ethyl alcohol for 3 min. followed by more washing with sterilized distilled water (Ash and Allen, 1948). The nodule was crushed with sterilized glass rod in a small aliquot of sterilized water and diluted for obtaining clear and distinct colonies. Congo red yeast-extract mannitol agar medium (CRYMA) was used for the isolations. The constituents of the medium were as follows:

Mannitol	10 g
Yeast extract	1.0 g
NaCl	0.1 g
K_2HPO_4	0.5 g
Mg SO_4 $7H_2O$	0.2 g
Agar agar	20 g
Distilled water	1000 g
Congo red	2.5 ml of 1% solution

One ml of the dilution was added to each petriplate containing 15 ml of CRYMA medium.The petriplates were incubated at 30°C(±2) for one week. Distinct white, translucent, glistening elevated colonies of *Rhizobium* sp. which developed on the media in the petriplates were picked up and purified by reculturing.

Pure culture of *Rhizobium* sp.

Yeast extract mannitol agar (YMA) was used for pure culturing of *Rhizobium* sp. (Fred *et. al.*, 1932).The composition of YMA used was as follows:

K_2HPO_4	0.5 g
Mannitol	10 g
$MgSO_47H_2O$	0.2 g
NaCl	0.1 g
Yeast extract	0.4 g
Agar-agar	15 g
Distilled water	100 ml
pH	6.8-7.0

The medium was autoclaved at 15 lb p.s.i. (120°C) for 20 minutes. The medium was poured in sterilized petriplates. After solidification of the medium the tested *Rhizobium* sp. isolate from the black gram was inoculated in the plates in aseptic conditions at laminar flow bench. After inoculation, the petriplates were kept at 30°C (±2) in an incubator for one week after which colonies developed in the plates. The bacterium was transferred in culture tubes containing YMA medium.

Soil based culture

For artificial inoculation of black gram, the soil basd culture of *Rhizobium* sp. was prepared and seed dressing was done prior to sowing the seeds according to the treatments.

For culturing root nodule bacterium in soil, a mixture of field soil and compost in the ratio of 1:1 was prepared. One Kg of the soil-compost mixture was autoclaved and the pH was maintained at 7 by mixing 10 g $CaCO_3$. After that 10 g sugar (commercial) and 0.5 g K_2HPO_4 were added to the soil-compost mixture. Then pure culture

Rhizobium sp. grown on YMA was mixed thoroughly in the mixture. This mixture of *Rhizobium* sp. soil and compost was used for inoculating the seeds of black gram.

Inoculation with root nodule bacterium

Incoulation of the seeds of black gram was done prior to their sowing by using soil-based culture of *Rhizobium* sp. Commercial sugar and water were added to the soil-based culture with thorough mixing. The seeds were treated with this mixture followed by drying the seeds in shade for about half an hour before sowing.

Plant culture and treatment

For studying the effects of SO_2 and O_3 seeds of the test plant, black gram (*Vigna mungo* (L.) Hepper) cv. Type 9 Pantnagar were surface sterilized for 2 min. in 0.1% $HgCl_2$ solution. Seeds were sown in 72 clay pots (9cm diam.) having sterilized field soil (66% sand, 24% silt, 8% clay, 20 M., pH 7.5). The sowing was done in 4 sets. Each set consisted of 18 pots. The first set of pots contained only plants i.e. without any root symbiont, *G. caledonicum* or *Rhizobium* (uninoculated). In the second set, plants were raised from seeds, treated *Rhizobium* (single inoculation) in the third set, plants were inoculated with *G. caledonicum* (single inoculation). In the fourth set of plants were inoculated with both *Rhizobium* sp. and *G. caledonicum* (dual inoculation).

The plants of the experiments, according to the designated treatments, were exposed intermittently to different doses of gaseous of air pollutants, sulphur dioxide (SO_2) and ozone (O_3) in exposure chambers. The following was the pattern of treatments for the two (SO_2 and O_3) air pollutants.

1. Sulphur dioxide

Two concentrations of SO_2 (0.05 and 0.1 ppm) were used for exposure of the plants designated to receive the SO_2 treatments.

I. Sulphur dioxide

(a) Control set

Plant (uninoculated)

Plant + *Rhizobium* sp. (single inoculation)

Plant + *G. caledonicum* (single inoculation)

Plant + *G. caledonicum* + *Rhizobium* sp. (dual inoculation)

(b) Exposed set

(i) Plant + 0.05 ppm SO_2

Plant + *Rhizobium* sp. + 0.05 ppm SO_2

Plant + *G. caledonicum* + 0.05 ppm SO_2

Plant + *G. caledonicum* + *Rhizobium* sp.+ 0.05 ppm SO_2

(ii) Plant + 0.1 ppm SO_2

Plant + *Rhizobium* sp+ 0.1 ppm SO_2

Plant + *G. caledonicum* + 0.1 ppm SO_2

Plant + *G. caledonicum* + *Rhizobium* sp+ 0.1 ppm SO_2

Plant of set (i) and (ii) were exposed for 3 h on alternate days.

2. Ozone

Plant were exposed to ozone using two sequences of its concentrations i.e. (i) 0.02-0.05-0.02 ppm O_3,(ii).0.05- 0.1-0.05 ppm O_3. Exposure durations for each concentration in the both sequences also varied as given below in the treatments.

II.Ozone

(a) Control Set

Plant (uninoculated)

Plant + *Rhizobium* sp. (single inoculation)

Plant + *G. caledonicum* (single inoculation)

Plant + *G. caledonicum* + *Rhizobium* sp. (dual inoculation)

(b) Exposed set

(i) Plant + 0.02-0.05-0.02 ppm O_3

Plant + *Rhizobium* sp. + 0.02-0.05-0.02 ppm O_3

Plant + *G. caledonicum* + 0.02-0.05-0.02 ppm O_3

Plant + *G. caledonicum* + *Rhizobium* sp.+ 0.02-0.05-0.02 ppm O_3

Plants were exposed on alternate days. Total exposure was given continuously for 7h with a change of concentration and duration of exposure i.e. 0.02 ppm for 2 h to start with, followed by 0.05 ppm for 3 h and again 0.02 ppm for 2 h.

(ii) Plant + 0.05-0.1-0.05 ppm O_3

Plant + *Rhizobium* sp.+ 0.05-0.1-0.05 ppm O_3

Plant + *G. caledonicum* + 0.05-0.1-0.05 ppm O_3

Plant + *G. caledonicum* + *Rhizobium* sp.+ 0.05-0.1-0.05 ppm O_3

Like exposed set (i) exposure was the also given continuously for 7 h with a change of concentration and duration of exposure i.e. 0.05 ppm for 2 h in the begining, 0.1 ppm for 3 h, and again 0.05 ppm for 2h.

EXPOSURE SYSTEM

Exposure chamber

The exposure system consisted of 3 chambers each of dimensions 90 x 90 x 120 cm. Two chambers were used for air pollutant exposures and the third one as a control (ambient air). Each chamber was made of transparent glass fibre, with an exhaust duct at the top and double-walled bottom, the upper wall being perforated while the lower wall was equipped with a blower assembly. A fumigation controller regulated the voltage supply to the blower and displayed it on a meter fitted to the chamber. The chambers had a movable front door and were horizontally partitioned by a meshed iron tray to provide additional space for the placement of pots. The exhaust duct (20 x 20 cm) of the exposure chamber were connected to a vertical exhaust pipe fitted in the roof of the glasshouse (Fig. 1).

Gas generation

Sulphur dioxide was generated in a generator which produced SO_2 gas by the action of sulphuric acid (H_2SO_4) on sodium sulphite (Na_2SO_3) under control reaction conditions. The amount of Na_2SO_3 and H_2SO_4 discharged from the reagent bottles mounted over the SO_2 generator were determined by collecting the solution dropping through capillary tube in a graduated cylinder for sometime and expressing the rate in ml /min. On the basis of flow rate or solution feeding rate, solutions of Na_2SO_3 and H_2SO_4 (10%) was prepared to produce required amount of SO_2 gas/min. On complete reaction 1M Na_2SO_3 produces 1 SO_2 or 126 mg Na_2SO_3 produces 64 mg SO_2 .

$$Na_2SO_3 + H_2SO_4 \text{-----} > SO_2 + Na_2SO_4 + H_2O$$

Ozone

Ozone was generated by subjecting dry oxygen to the action of silent electric discharge in an apparatus called ozoniser

$$3O_2 \Leftrightarrow 2O_3$$

The concentration of SO_2 (0.05 ppm and 0.1 ppm) and

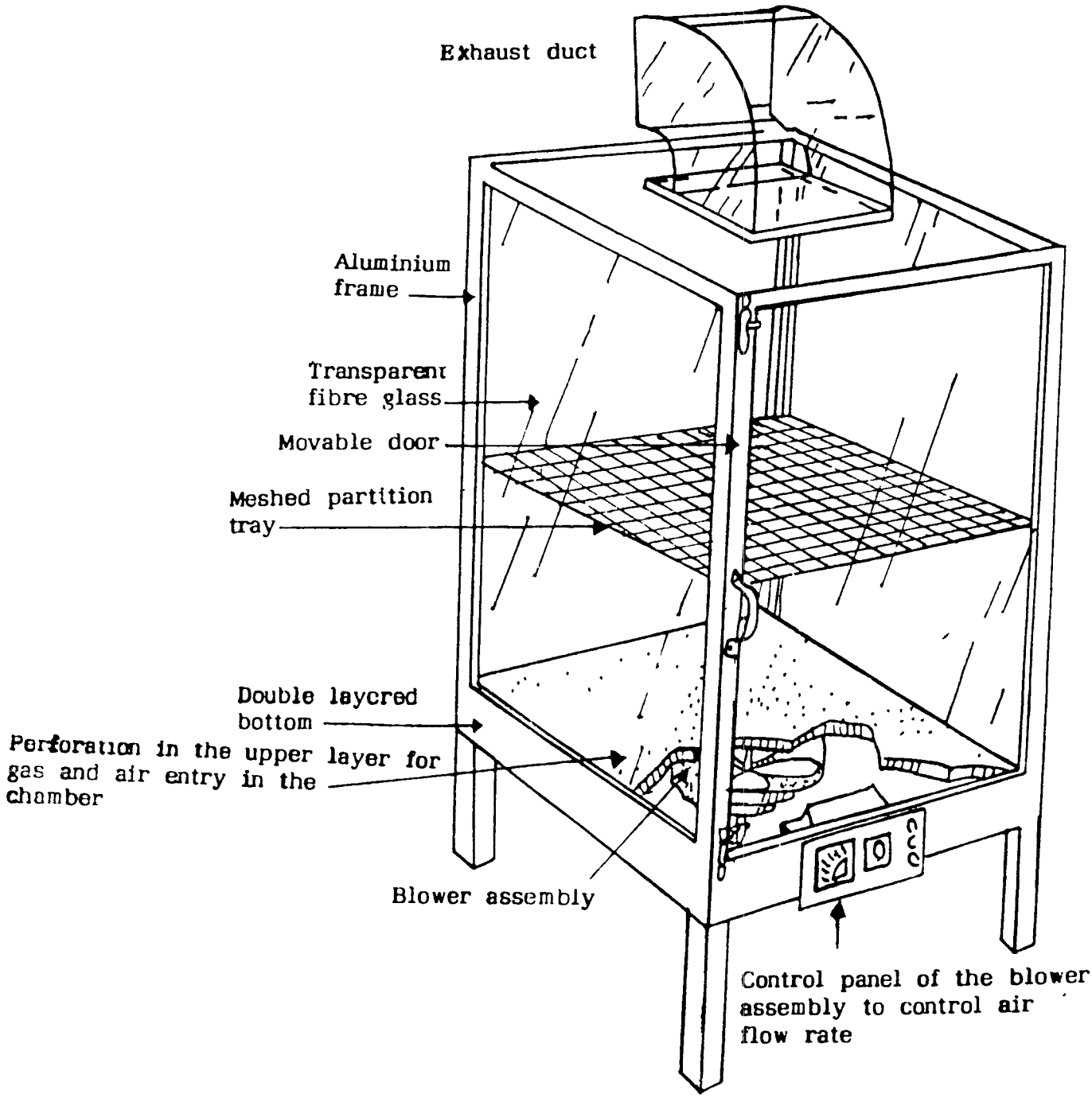


FIGURE 1

EXPOSURE CHAMBER

O_3 (0.02 ppm, 0.02-0.05-0.02 ppm and 0.05-0.1-0.05 ppm) was also determined by sampling the air using a portable air sampler (Kimoto Electricals, Japan) and was analysed by spectrophotometry (Anon., 1986). The outlet of the gas generator was connected to the gas inlet nozzles provided in the blower housing of the exposure chamber.

Treatment with the gaseous air pollutants (SO_2 and O_3)

Treatment with SO_2

All set of black gram (22-day-old plants) designated to be treated with SO_2 were exposed for 3 h on alternate days to 0.05 ppm and 0.1 ppm till the termination of the experiment (80 days). Total 29 exposures were made in the experiment. After every exposure, pots were again transferred to glasshouse.

Treatment with O_3

Twenty two-day-old plants of black gram of the exposed sets were treated with O_3. Plants were exposed to two concentration sequences 0.02-0.05-0.02 ppm and 0.05-0.1- 0.05 ppm on alternate days . The exposures were continued for 7 h with a change of concentration and duration of exposure i.e. (1) 0.02 ppm for 2 h, 0.05 ppm for 3 h and again to 0.02 ppm for 2 h (2) 0.05 ppm for 2 h, 0.1 for 3 h and again 0.05 ppm for 2 h.

All sets were exposed, according to the schedule described above, both for SO_2 and O_3 till the termination of experiment (80 days). Total 29 exposures for each concentration were made for the crop. After every exposure, pots were again transferred to glasshouse. In both the experiments, each treatment was replicated six times and pots were arranged in complete randomized block design (CRBD) in the glassshouse.

Fly ash

For stuying the effect of amendment of soil with fly ash, clay pots of 9 cm diam. were filled with a mixture of soil and fly ash and autoclaved. Fly ash for the experiment was collected from a thermal power plant located at Kasimpur, 15 Km away from the Aligarh Muslim

University Campus. The power plant uses bitumunous type of coal. Surface sterlized seeds of black gram were sown in sterilized clay pots containing a mixture of soil and fly ash in different ratios (V/V). Different levels (V/V) of fly ash used in the study (20,40,60,80,100%) were obtained by adding fly ash to the sandy loam field soil (66% sand, 24% silt; 8% clay, O.M 2%, pH 7.5) in desired amounts. Pots were divided into four sets each having 36 pots. In the first set of pots, the plants were kept uninoculated (control). Inoculation of symbionts (singly and in combination) were done in 3 sets. Inoculation with *Rhizobium* sp. (seed dressings) and *G. caledonicum* (1000 spores/pot) were done in same manner as described earlier.

In the experiment with fly ash following were the treatments.

(a) Unamended soil (0% fly ash)

Plant (uninoculated)

Plant + *Rhizobium* sp.

Plant + *G. caledonicum*.

Plant + *Rhizobium* sp. + *G. caledonicum*.

(b) Amended soil (fly ash level)

Plant + 20% fly ash

Plant + *Rhizobium* sp. + 20% fly ash

Plant + *G. caledonicum* + 20% fly ash

Plant + *Rhizobium* sp. + *G. caledonicum* + 20% fly ash

Plant + 40% fly ash

Plant + *Rhizobium* sp.+ 40% fly ash

Plant + *G. caledonicum* + 40% fly ash

Plant + *Rhizobium* sp. + *G. caledonicum* + 40% fly ash

Plant + 60% fly ash

Plant + *Rhizobium* sp.+ 60% fly ash

Plant + *G. caledonicum* + 60% fly ash

Plant + *Rhizobium* sp. + *G. caledonicum* + 60% fly ash

Plant + 80% fly ash

Plant + *Rhizobium* sp.+ 80% fly ash

Plant + *G. caledonicum* + 80% fly ash

Plant + *Rhizobium* sp. + *G. caledonicum* + 80% fly ash

Plant + 100% fly ash

Plant + *Rhizobium* sp.+ 100% fly ash

Plant + *G. caledonicum* + 100% fly ash

Plant + *Rhizobium* sp. + *G. caledonicum* + 100% fly ash

Each treatment was replicated six times and pots were arranged in complete randomised block design (CRBD) on glasshouse benches.

Parameters

After termination of the experiments, following parameters were determined for each treatment of the experiments.

Root and shoot lengths

Fresh and dry weight of shoot and root

Yield (number of pods per plant and number of seeds per pod)

Root colonization by the VAM fungus

Number of VAM spores/100 g of soil

Number and dry weight of nodules/root system

Chlorophyll content of leaf

Nitrogen and phosphorus content of roots and shoots

Protein content of seeds.

Plant growth and yield

After termination of the experiment, for determining length and fresh weight of shoot and root, plants of each treatment were taken out from the pots and soil particles adhering of roots were removed with tap water and properly labelled and brought to the laboratory. In the laboratory, lenghts of shoot and root were measured by measuring tape and fresh weights of shoot and root were determined by physical balance. For determining dry weights of root and shoot, plants from each treatment were wrapped in a blotting sheet, labelled and dried in a hot air oven at 60°C for 24 h and weighed. Number of pods/plant and number of seeds/pod were counted before dry weight was taken, to determine the yield in each treatment.

Root colonization by the VAM fungus and estimation of VAM spores from the soil

At the termination of the experiments, root colonization of the plants by the VAM fungus was assessed. Three soil cores were taken with the help of an auger (2.5 cm diam.) from each pot of the treatments inoculated with *G. caledonicum*. The three soil cores from each pot of the each treatment were mixed which was considered as one sample. Soil of each sample was suspended in water and roots were retained on a 100 mesh sieve. Roots were cut 1 cm long. Root pieces (1 cm long) were cleared by 10% KOH and alkaline H_2O_2 and stained by Trypan blue (0.05% in lactophenol) solution according to the procedure given by Phillips and Hayman (1970).

Percentage of root colonization was determined by slide method (Giovanetii and Mosse, 1980). Ten root samples were mounted on each glass slide, selected randomly from stained samples and examined microscopically (25x) for root colonization. One hundred to one hundred and fifty (100-150) root segments from each sample were used for the assessment. The presence or absence of colonization in each root segment was recorded and result was expressed as

percentage of root colonized. The root colonization (mycorrhizal infection in the roots) was calculated as follows:

$$\text{VAM association} = \frac{\text{No. of mycorrhizal segments}}{\text{Total no. of segments examined}} \times 100$$

Estimation of spores

For estimating the spores of *G. caledonicum* in the same soil samples used for assessment of root colonization, spores were isolated from the soil of the treatments inoculated with VAM fungus by wet sieving and decanting method (Gerdemann and Nicolson, 1963). Repeated washings with Ringer's solution were done to separate the spores from soil matter. The suspension thus obtained was made upto 50 ml. The spores were counted in 1 ml of suspension in a nematode counting dish under the stereoscopic microscope. The final number of spores/100 g soil was calculated.

Root nodulation

Before drying the plants for determining their dry weight, number of nodules per root system in the treatments having *Rhizobium* sp. inoculation were counted. Then after nodules were detached from the roots and placed separately for each treatment in labelled petridishes. The petridishes contianing the nodules were kept in an hot air oven at 60°C and dry weight of the nodules were determined. This dry weight was added to the root dry weight of respective treatments.

Plant analysis

Chlorophyll content of leaves, nitrogen and phosphorus contents of root and shoot, protein content of seeds were estimated from the plants of each treatment.

Chlorophyll estimation

Chlorophyll content of leaves of black gram from different treatments of the experiment was estimated. Leaves of different ages (tender to old leaves) were collected from all the sets of black gram plants of each treatment. One gram of interveinal region of the leaves was ground in 40 ml of 80% acetone with the help of mortar and pestle.

The suspension was decanted in buchner funnel having two Whatman paper no.1. The filtration was done with the help of suction pump. The residue was ground thrice adding with 30, 20 and 10 ml of acetone respectively. The suspension was decanted in buchner funnel and filtered in vacuum. At last mortar and pestle were rinsed with 80% acetone, transferred in buchner funnel and filtered in vacuum. The filtrate were transferred in 100 ml volumetric flask and the volume was made upto capacity. The tansmittance were read at 645,663 and 635 nm at spectrophotometer. The chlorophyll a, b and total chlorophyll were calculated accordingly by using optical density (O.D.) i.e. by using % transmittance (Machinney,1941).

$$\text{Chl.a in fresh tissue} = 12.7(\text{O.D.663}) - 20.6(\text{O.D.645}) \times \frac{V}{1000W}$$

$$\text{Chl.b in fresh tissue} = 22.9(\text{O.D.645}) - 4.68\ (\text{O.D.663}) \times \frac{V}{1000W}$$

$$\text{Total chl.in fresh tissue} = 20.2\ (\text{O.D. 645}) + 8.02(\text{O.D. 663}) \times \frac{V}{1000W}$$

Nitrogen and phosphorus estimation from shoot and root

For estimation of nitrogen and phosphorus, shoot and root samples were digested as given below :

Digestion of shoot and root samples

Shoot and root samples of plants from various treatments of the experiments were digested first according to the following method.

100 mg of oven dried shoot and root powder were transferred in 50 ml kjeldahl flask, then 2 ml of chemically pure H_2SO_4 was added and flasks were heated on kjeldahl assembly for about 2 h, till the dense fume had given-off and the contents had turned black. The 0.5ml of pure 30% H_2O_2 was added after 15 minutes of cooling. Heating was done again till the colour was changed into light yellow. It was heated again for half an hour and flasks were cooled for 10 min for getting the extract clear. Then 3-4 drops of 30% H_2O_2 were added dropwise

followed by heating for 15 min. After that digested material was transferred in 100 ml volumetric flask with 3-4 washing and used for estimating N and P etc. present in the shoots and roots (Linder, 1944; Lundegardh,1951).

Nitrogen from shoot and root

Prior to estimating the N content present in the digested material of shoot and root, standard curve was drawn by the following procedure:

0.236 g of ammonium sulphate was dissolved in 100 ml of solution, then 0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9,1.0 ml solutions were poured in test tubes respectively. The volume was made upto 5 ml in each test tube by adding distilled water. A control was also run side by side. After that 0.5 ml Nesseler's reagent was added followed by 5 ml of distilled water. The percentage transmittance was read at 525 nm on spectrophotometer on developing yellow organe colour after half an hour. Then a curve was drawn on graph between concentration and O.D.

Estimation

10 ml of aliquot (digested shoot and root material) was taken in 100 ml volumetric flask and 2 ml, 2.5 N NaOH was added to neutralise the excess amount of acid present. Then 1 ml of 10% sodium silicate was added to prevent turbidity and volume was made upto capacity 5 ml of aliquot was taken in 3 test tubes followed by addition of 0.5 ml of Nesseler's reagent with shaking. Then 10 ml volume was made by adding distilled water. After waiting for 5 min the % transmittance was read at 525 nm. Concentrations were read from standard graph by using O.D. (Linder, 1944).

Phosphorus

At first a standard curve was prepared. Different concentrations of KH_2PO_4 solution ranging from 0.1 to 1 ml were taken in 10 separate test tubes and the volume of each test tube was maintained upto 5 ml. Then 1 ml ammonium molybdic acid and 0.4ml of 1 amino-2-nepthol-4-sulphonic acid were added in each test tube

followed by making the volume upto 10 ml with distilled water. After half an hour % transmittance was read at 625nm. Then standard curve was drawn between concentration and O.D.

Estimation

5 ml of aliquot (digested shoot and root) were taken in three test tube to which 5 ml of distilled water was added. After that 1 ml of ammonium molybdic acid was added, with shaking, followed by addition of 0.4 ml 1amino-2-nepthol-4-sulphonic acid. The control was also run side by side. Percentage transmittance was read at 625 nm after half an hour. Concentrations were read from standard graph by using O.D. (Fiske and Row, 1925).

Protein estimation

The protein content of the seeds of black gram was estimated by the method given by Lowry *et al.*, (1951).

Following reagents were prepared for estimation soluble and insoluble proteins present in the seeds:

Reagent A-2% sodium carbonate in 0.1 N NaOH in ratio of 1:1.

Reagent B-0.5% $CuSO_4$ in 1% sodium tartrate in ratio of 1:1.

Reagent C-50 ml reagent A+1 ml reagent B (Alkaline $CuSO_4$). (Carbonate $CuSO_4$ soln.) .

Reagent D-50 ml of 2% sodium carbonate + 1 ml reagent B .

Reagent E-Folin's reagent diluted to make 1N (Diluted Folin's reagent).

Standard curve

Before actual estimation, a standard curve was prepared by dissolving 40 mg of egg albumin in 0.1N NaOH solution, the volume of which was made upto 100 ml by adding distilled water. From this solution, aliquots of 0.1 ml to 1 ml were taken in 10 test tubes. Reagent A was nowadded to the test tubes. After 10 min 0.5 ml reagent E was

added to the test tubes. The % transmittance were read at 660 nm and standard curve was drawn between O.D. and concentration.

Soluble protein

50 mg dry powder of seeds was ground with 5 ml of double distilled water with mortar and pestle. Then water extract was decanted in centrifuge tube for centrifugation at 4000 rpm for 10 min. The supernatant was collected in 50 ml volumetric flask and residue was retained in centrifuge for estimating insoluble proteins. After making the volume upto 50 ml by adding DDW, 1 ml of water extract was transferred in a 10 ml test followed by addition of 5 ml of reagent C. After mixing, solution was left as such for 10 minutes. Then 5 ml of reagent E was added and mixed immediately. The control was run along with experimental set. Percent transmittance was read at 660 nm after half an hour. The corresponding protein content were measured, by using the standard curve.

Insoluble protein

The residue retained in the centrifuge tube was used for insoluble protein estimation. 5ml of 5% tricholoracetic acid was added to the residue with shaking. After half an hour it was cetrifuged at 4000 rpm to 10 min. The supernatant was discarded. 5ml of 1N NaOH was added in the residue with vigorous shaking. After half an hour it was again centrifuged and supernatant was collected in 50 ml volumetric flask and volume was made upto 50 ml within 1N NaOH.

1 ml of the solution was taken in test tube with 5 ml of reagent D followed by mixing. After 10 min. 0.5 ml of reagent E was added with immediate mixing. 1N NaOH was used in control. Percent transmittance were read at 660 nm after 30 min. The protein content was calculated by using the standard curve. By adding soluble and insoluble protein, total protein was calculated.

Statistical Analysis

Experiments were conducted in complete randomized block design, but while analysing the data, this was further extended to split the factors and analysed by extending the method adopted by Panse and

Sukhatme (1954). In the experiment, black gram was selected as factor one and symbiont treatments (pollutant, *Rhizobium* sp., *G. caledonicum*) as factor two. The data obtained were subjected to analysis of variance (ANOVA) to determine significance and C.D. was calculated at P=0.05 to separate the means of replicates for significance. The ANOVA model adopted for the analysis of variance comes as follows, where

R = Replicates

F_1= Factor one

F_2= Factor two

d F = Degree of freedom

SS = Sum of squares

MS = Mean of squares

Fcal.= F value calculated

F Tab. = F value tabulated.

Variable	d.F.	SS	MS	F.cal	F.Tab
R	R-1	Calculated as per procedure	SS/RdF	RMS/EMS	RdFVsEdF
F_1	(F_1-1)		SS/F_1dF	F_1MS/EMS	F_1dFVsEdF
F_2	(F_2-1)		SS/F_2dF	F_2MS/EMS	F_2dFVsEdF
Interaction i.e. F1 x F_2	(F_1-1) x (F_2-1)		SS/F_1xF_2dF	F_1xF_2MS/EMS	F_1/F_2dFVsEdF
Error	(R-1)(F_1-1)+(R-1)F_1(F_2-1)		SS/EdF		
Total	(RxF_1xF_2)-1				

Standard error (SE) was calculated as follows prior to calculation of the CD. (Critical difference).

$$\text{SE for } F_1 = \sqrt{\frac{2EMS}{R \times F_2}}$$

$$\text{SE for } F_2 = \sqrt{\frac{2EMS}{R \times F_1}}$$

$$\text{SE for } F_1 \times F_2 = \sqrt{\frac{2EMS}{R}}$$

CD was calculated at P=0.05 with the help of calculated S.E. as follows.

CD for F_1 = SE for F_1 xt value at 5%

CD for F_2 = SE for F_2 xt value at 5%

CD for $F_1 \times F_2$ = S.E. for $F_1 \times F_2$ xt value at 5% .

In this way, three CD were calculated, significancy and non-significancy of CD was calculated with help of ANOVA table if F cal was found to be greater than F. Tab., the data were considered as significant and a vice-versa.

RESULTS

SULPHUR DIOXIDE

Plant growth

Plant growth of black gram was improved by inoculation of the plants with the root symbionts. All the considered plant growth parameters (lengths and fresh and dry weights of shoot and root) showed an increase in plants inoculated with the root symbionts, *Rhizobium* sp. and *G.caledonicum*, either singly (single inoculation) or in combination (dual inoculation). The considered plant growth parameters were greater in dual inoculated plants than in plants inoculated with either of the symbionts. The VAM was more effective in increasing the plant growth parameters than the root nodule bacterium (Table 1, Fig.2,3;Plate I).

Intermittent exposure of the plants to SO_2 suppressed plant growth variously in different treatments. Shoot and root lengths of the plants were reduced (P=0.05) at both concentrations of SO_2 (0.05 and 0.1 ppm) as compared to unexposed plants. Exposure of uninoculated plants to SO_2 concentrations caused significant reduction in shoot lengths, compared to unexposed plants. Shoot length of uninoculated unexposed plants, were less in comparison to nodulated, mycorrhizal and dual inoculated exposed plants. Dual inoculated plants showed highest shoot length followed by mycorrhizal and nodulated plants. Single inoculated plants (with either of the symbionts) suffered suppressions by SO_2 exposures in their plant lengths than single inoculated unexposed plants. Plants inoculated with *Rhizobium* sp. (nodulated plants) showed 8.1 and 14.4% suppressions and those with *G.caledonicum* (mycorrhizal plants) showed 9.9 and 11.4% , respectively as compared to unexposed plants inoculated with respective root symbionts. Exposure of dual inoculated plants to SO_2 also caused reduction in their shoot length. Percentage reductions at both the SO_2 concentrations were 7.1 and 7.4, respectively as compared to the unexposed dual inoculated plants (Table 1 ;Fig. 2).

Root lengths were also suppressed by SO_2 exposures in all the treatments. Root length of uninoculated exposed plants was found to be reduced in comparison to uninoculated unexposed plant. In nodulated,

Table 1. Effect of SO_2 on length and fresh weight of shoot and root of black gram plants inoculated with root symbionts.

Treatment		SO_2 (ppm)					
		Length (cm)			Fresh weight(g)		
		0.0	0.05	0.1	0.0	0.05	0.1
Plant (Black gram without symbionts)	S	35.10	32.13	30.07	7.05	5.8	4.65
	R	8.16	7.01	6.05	1.14	0.96	0.68
Plant + *Rhizobium* sp.	S	37.21	34.16	31.95	8.54	7.36	5.80
	R	9.8	7.83	6.86	1.44	1.043	0.80
Plant + *Glomus caledonicum*	S	37.83	34.95	33.5	9.3	8.36	7.61
	R	9.8	8.04	7.7	1.81	1.29	0.99
Plant + *Rhizobium* sp. *G. caledonicum*	S	41.13	38.18	38.08	12.01	10.1	90.1
	R	18.0	11.0	10.1	2.18	1.55	1.46

	C.D. (P=0.05)			
	Length		Fresh weight	
	S	R	S	R
Treatments	0.6079	0.5328	0.13850	0.11027
SO_2	0.713	0.61531	0.15993	0.12733
Interaction	1.214	1.0657	0.27700	0.22055

S=Shoot, **R**=Root

Each value is mean of six replicates.

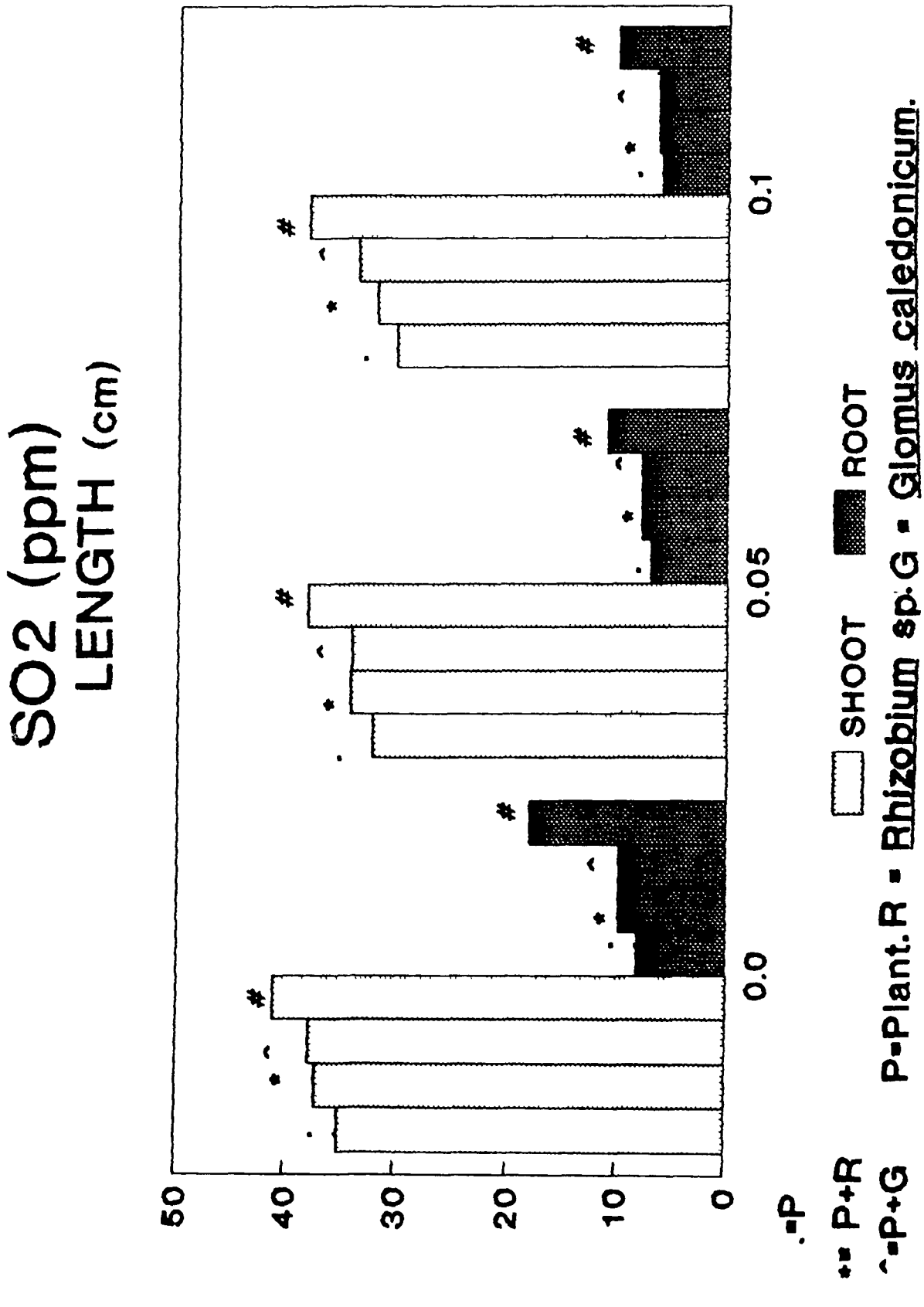

SO2 (ppm)
LENGTH (cm)
50
40
30
20
10
0
0.0
0.05
0.1
SHOOT
ROOT
.=P
+= P+R
^=P+G
#= P+R+G
P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.2

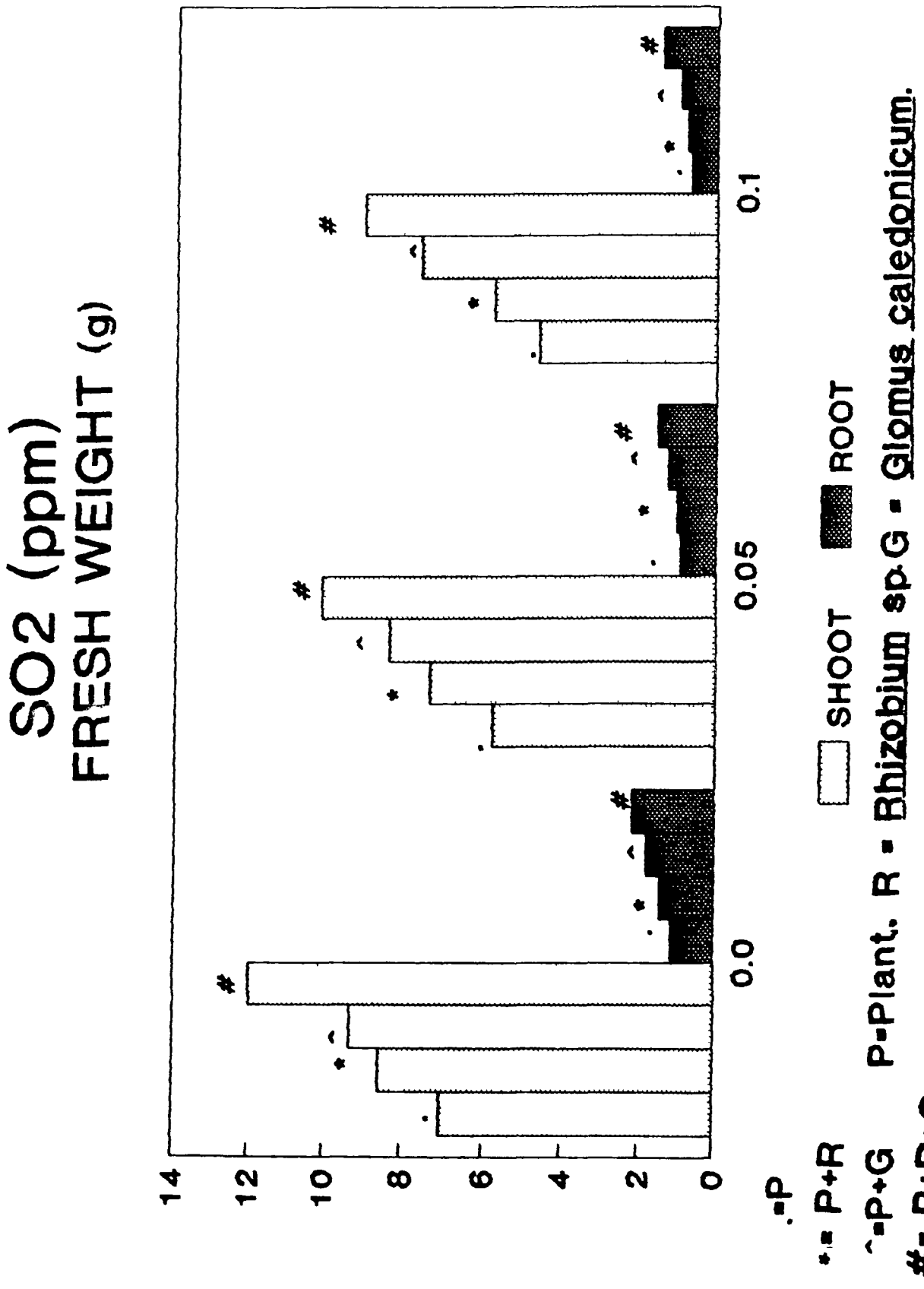
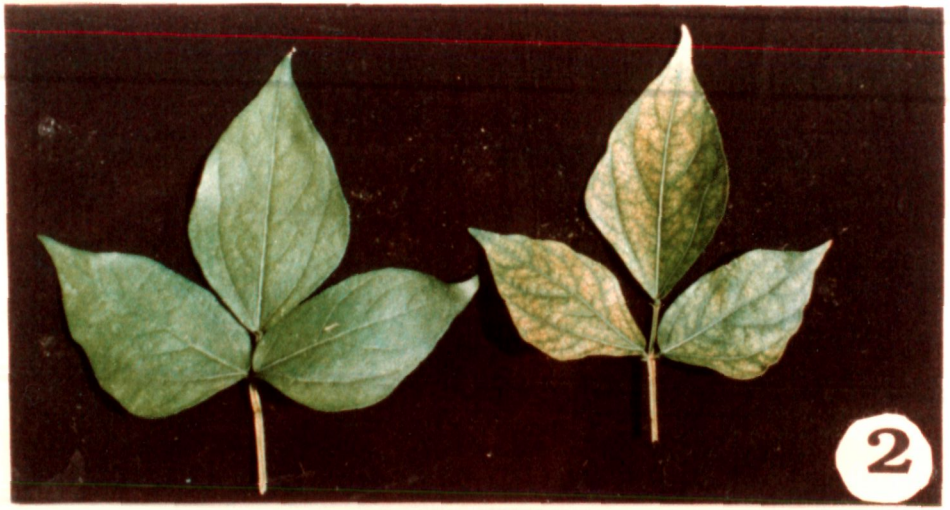

SO2 (ppm)
FRESH WEIGHT (g)
14
12
10
8
6
4
2
0
0.0
0.05
0.1
SHOOT
ROOT
P=Plant, R = Rhizobium sp.G = Glomus caledonicum.
.=P
*= P+R
^=P+G
#= P+R+G

Fig.3

Plate No. I :- Effect of SO_2 exposure on black gram plants uninoculated and inoculated with root symbionts.

1. Plants exposed at 0.05 ppm concentration SO_2 .
2. Leaves from unexposed and exposed plants (0.05 ppm SO_2).
3. Plants exposed at 0.1 ppm concentration SO_2 .
4. Leaves from unexposed and exposed plants (0.1 ppm SO_2).


1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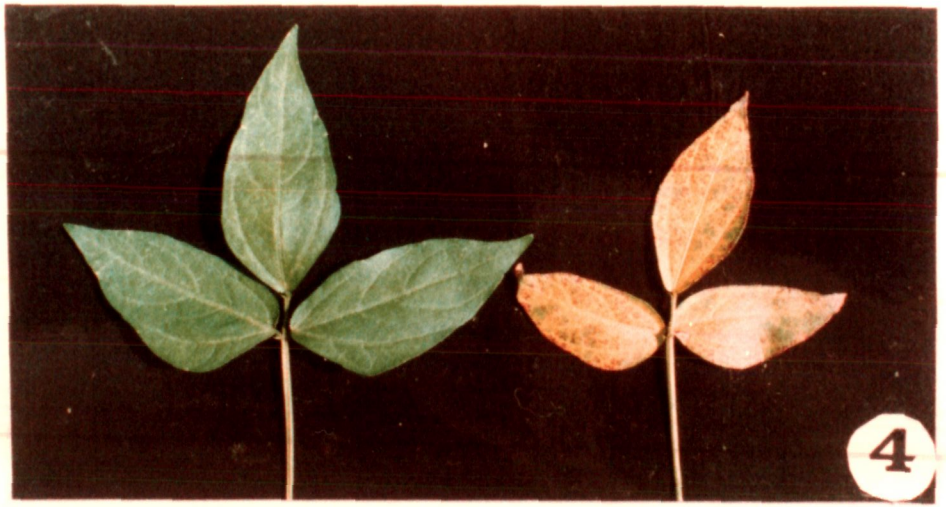
3


4

mycorrhizal and dual inoculated plants exposed to SO_2 root lengths were significantly greater than uninoculated unexposed plants. However, root lengths of nodulated, mycorrhizal and dual inoculated plants were suppressed, compared to their respective unexposed inoculated controls. The nodulated plants showed a suppression of root length by 20.1 and 34.5% and mycorrhizal plants 17.9 and 21.4% and dual inoculated 38.8 and 43.8% as compared to their respective unexposed inoculated controls at 0.05 and 0.1 ppm SO_2,respectively (Table 1; Fig.2).

Like lengths, fresh weights of root and shoot were also suppressed at both concentrations of SO_2. Shoot fresh weight of uninoculated exposed plants were significantly reduced in comparison to uninoculated unexposed plants.Nodulated, mycorrhizal and dual inoculated plants even after SO_2 exposures showed greater shoot fresh weight than uninoculated unexposed plants.Shoot fresh weight of dual inoculated exposed plants was greater by 41.2%,compared to uninoculated unexposed plants.Shoot fresh weight of nodulated,mycorrhizal and dual inoculated plants,were significantly suppressed (P=0.05) when exposed to SO_2,in comparison to their respective controls.Like shoot fresh weight,exposure of uninoculated plants to SO_2 concentrations resulted in significant reduction,in their root fresh weight compared to uninoculated unexposed plants. Significant reductions also occurred in nodulated,mycorrhizal and dual inoculated plants by SO_2 exposures at both concentrations in comparison to their respective controls. Single inoculated (with either of symbionts) and dual inoculated (with both symbionts) exposed plants, though suffered suppression in root fresh weight but their root fresh weights were still greater than uninoculated unexposed plants. Root fresh weight of dual inoculated exposed plants was 47.7% greater than uninoculated unexposed plants (Table 1; Fig.3).

Dry weight of uninoculated exposed plants was also reduced significantly at both concentrations of SO_2, compared to unexposed uninoculated plants. Nodulated, mycorrhizal and dual inoculated plants exposed to SO_2 concentrations also exhibited reductions in their shoot and root dry weights in comparison to their respective controls. In shoot dry weight percentage reduction was 33.8 and 47.6 in nodulated,48 and 61.4 in mycorrhizal and 37.8 and 55.9 in dual inoculated at 0.05 and 0.1 ppm of SO_2 repectively .Dry weight of root and shoot of single inoculated and dual inoculated exposed plants were still significantly greater than unexposed (to either concentration of SO_2) plants

(Table 2; Fig.4).

Yield

Yield of black gram,assessed on the basis of pod number per plant and seed number per pod, of dual inoculated plants (*Rhizobium* sp. and *G.caledonicum*) and single inoculated plants (either by *Rhizobium* sp. or *G.caledonicum*) showed an increase, compared to uninoculated plants. Exposure of the plant to both concentrations of SO_2 did not cause significant reduction in pod or seed number in all the treatments (Table 2; Fig.5).

Leaf chlorophyll and seed protein

Intermittent exposures of the black gram plants adversely affected leaf chlorophyll and seed protein. Significant reduction occurred in leaf chlorophyll (chlorophyll a,chlorophyll b and total chlorophyll)at both (0.05 and 0.1 ppm) concentrations of SO_2. Significant reduction occurred in chlorophyll a, chlorophyll b and total chlorophyll in the leaves of uninoculated plants compared to uninoculated unexposed plants. Nodulated, mycorrhizal and dual inoculated plants exposed to SO_2 concentrations had greater chlorophyll than uninoculated unexposed plants.Dual inoculated exposed plants contained 11.5 and 9.20% chlorophyll a, 53.1 and 51.9% chlorophyll b and 34.8 and 33.2% total chlorophyll greater than uninoculated unexposed plants. Chlorophyll content of single or dual inoculated exposed plants was significantly less than inoculated unexposed plants (Table 3; Fig 6&7).

Seed protein of black gram was significantly reduced (P=0.05) by SO_2 exposures.Exposure of uninoculated plants caused 9.3 and 13.4% reductions at 0.05 and 0.1ppm respectively in seed protein compared to uninoculated unexposed plants.Seed protein in nodulated and mycorrhizal plants was significantly reduced by SO_2 exposures when compared to their respective controls. Similar reductions (P=0.05) occurred in dual inoculated plants which was 3.5% less at 0.05 and 5.5% less at 0.1 ppm SO_2 than dual inoculated unexposed plants.Though reduction in seed protein occurred in dual inoculated, nodulated, mycorrhizal plants exposed to SO_2 concentrations, they still contained significantly greater seed protein than uninoculated (with either of the symbionts) and unexposed (to either concentration of SO_2) plants (Table 3;Fig. 8).

Table 2. Effect of SO_2 on dry weight of shoot and root and yield of black gram plants inoculated with root symbionts.

Treatment		SO_2(ppm)						
		Dry weight(g)			Yield			
		0.0	0.05	0.1		0.0	0.05	0.1
Plant (Black gram	S	2.6	1.7	1.04	P.No.	7.41	5.0	4.68
without symbionts)	R	0.75	0.7	0.57	S.No.	18.9	7	3.8
Plant +	S	3.7	2.3	1.63	P.No.	7.83	6.3	4.83
Rhizobium sp.	R	0.89	0.82	0.6	S.No.	22.40	16.41	8.5
Plant + *Glomus*	S	5.0	2.6	1.93	P.No.	8.00	6.53	5.68
caledonicum	R	1.04	1.02	0.66	S.No.	31.48	23.6	17.9
Plant +	S	56.5	4.3	3.5	P.No.	10.83	8.6	8.0
Rhizobium sp.	R	1.31	1.1	1	S.No.	62.9	42.12	33.4
G. caledonicum								

	C.D. (P=0.05)			
	Dry weight		Yield	
	S	R	P.No.	S.No.
Treatments	0.05892	0.02669	1.60289	3.2887
SO_2	0.06804	0.030821	1.85086	3.7974
Interaction	0.11784	0.05338	(N.S.)	(N.S.)

S=Shoot, **R**=Root, **P.No.**=Pod number, **S.No.**=Seed number

Each value is mean of six replicates.


SO2 (ppm)
DRY WEIGHT (g)
70
60
50
40
30
20
10
0
0.0
0.05
0.1
SHOOT
ROOT
.=P
*= P+R
^=P+G
#= P+R+G
P=Plant. R = Rhizobium sp.G = Glomus caledonicum.

Fig.4

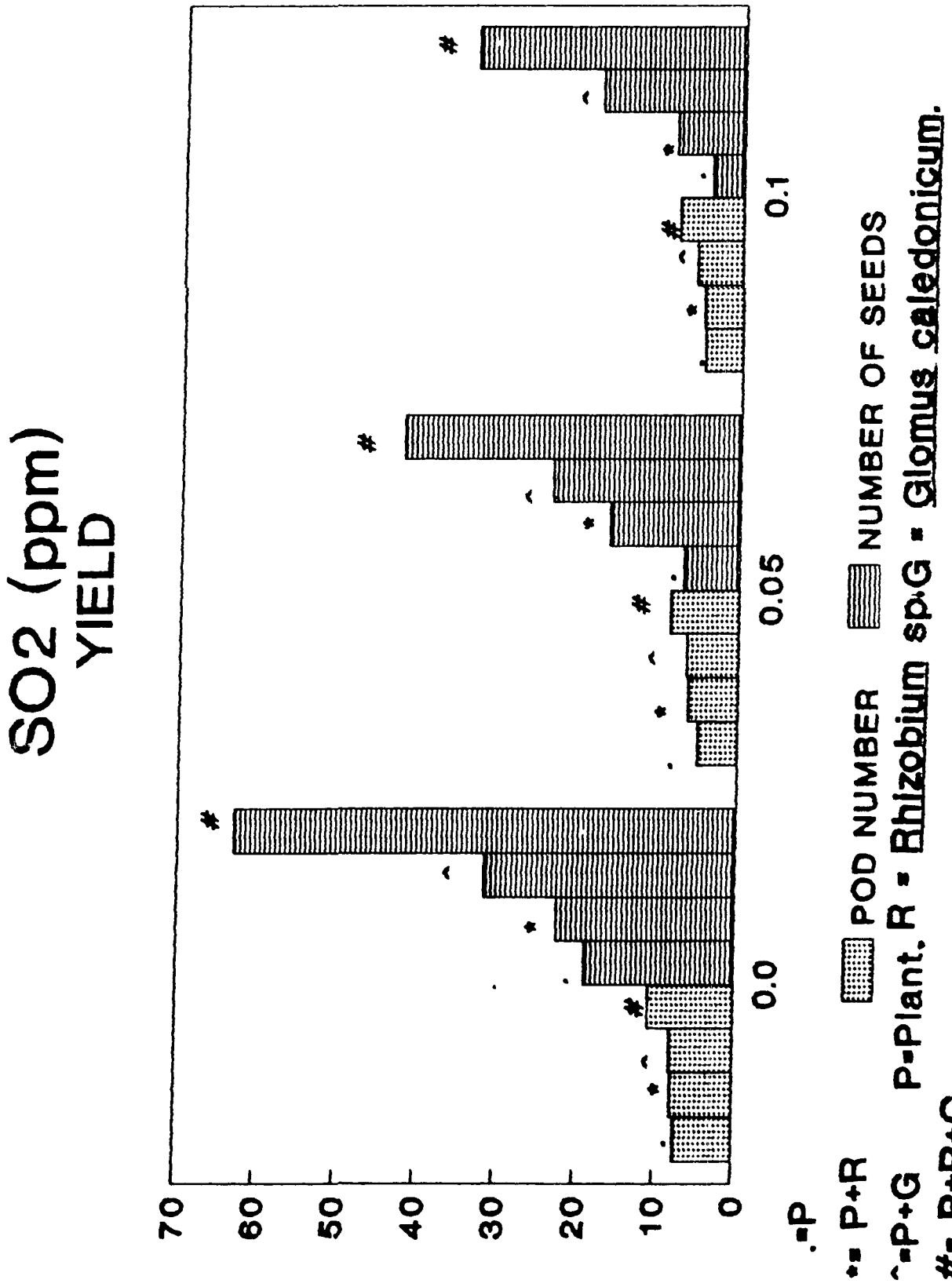

SO2 (ppm)
YIELD
70
60
50
40
30
20
10
0
0.0
0.05
0.1
POD NUMBER
NUMBER OF SEEDS
.=P
*= P+R
^=P+G
#= P+R+G
P=Plant, R = Rhizobium sp,G = Glomus caledonicum.

Fig.5

Table 3. Effect of SO_2 on chlorophyll content of leaf and seed protein of black gram plants inoculated with root symbionts.

Treatment		SO_2(ppm)					
		Chlorophyll (mg/g)			Protien(%)		
		0.0	0.05	0.1	0.0	0.05	0.1
Plant (Black gram	a	0.03727	0.03517	0.3282			
without symbionts)	b	0.08817	0.07850	0.07110	21.5	19.5	18.6
	T	0.12833	0.11500	0.1100			
Plant +	a	0.03810	0.3785	0.3760			
Rhizobium sp.	b	0.09662	0.9100	0.9000	22.01	21.9	21.3
	T	0.14483	0.13317	0.13067			
Plant + *Glomus*	a	0.04063	0.03900	0.03788			
caledonicum	b	0.13800	0.12517	0.11717	22.63	22.0	21.7
	T	0.15600	0.1550	0.1446			
Plant +	a	0.0440	0.04157	0.04070			
Rhizobium sp.	b	0.14000	0.13500	0.13400	24.67	23.8	23.3
G. caledonicum	T	0.17650	0.17300	0.17100			

	C.D. (P=0 05)			
	Chlorophyll			Protien
	a	b	T	
Treatments	0.0004172	0.000858	0.00087	0.8588
SO_2	0.0004817	0.000991	0.001004	0.99172
Interaction	0.0008344	0.001716	0.001739	1.7177

a=Chlorophyll a , **b**=Chlorophyll b, **T**=Total Chlorophyll

Each value is mean of six replicates.

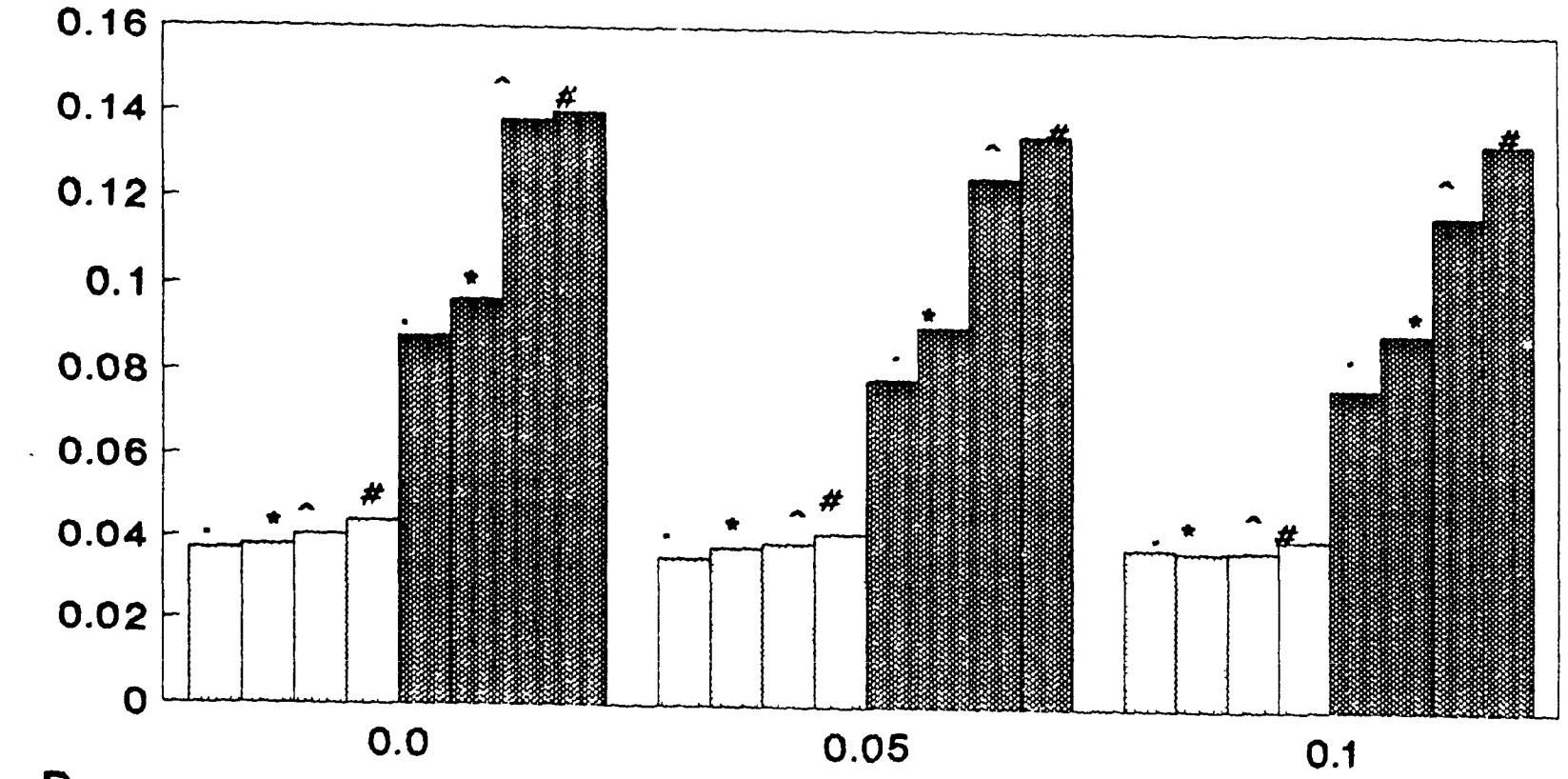

SO2 (ppm)
CHLOROPHYLL (a,b) (mg/g)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0.0
0.05
0.1
.=P
*= P+R
^=P+G
#= P+R+G
CHLOROPHYLL a
CHLOROPHYLL b
P=Plant.R = Rhizobium sp.G = Glomus caledonicum.

Fig.6

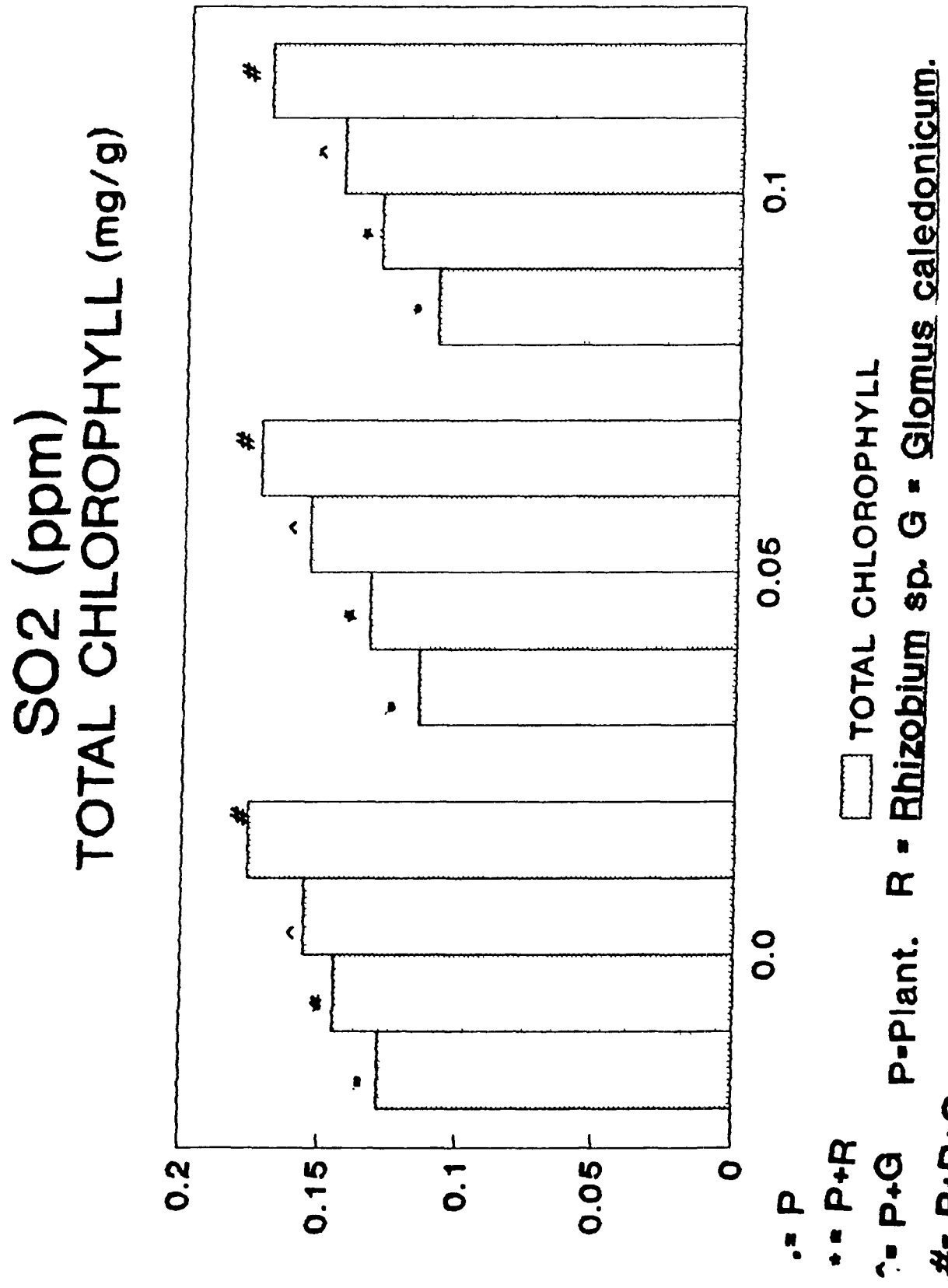

SO2 (ppm)
TOTAL CHLOROPHYLL (mg/g)
0.2
0.15
0.1
0.05
0
0.0
0.05
0.1
TOTAL CHLOROPHYLL
P=Plant. R = Rhizobium sp. G = Glomus caledonicum.
= P
= P+R
= P+G
#= P+R+G

Fig.7

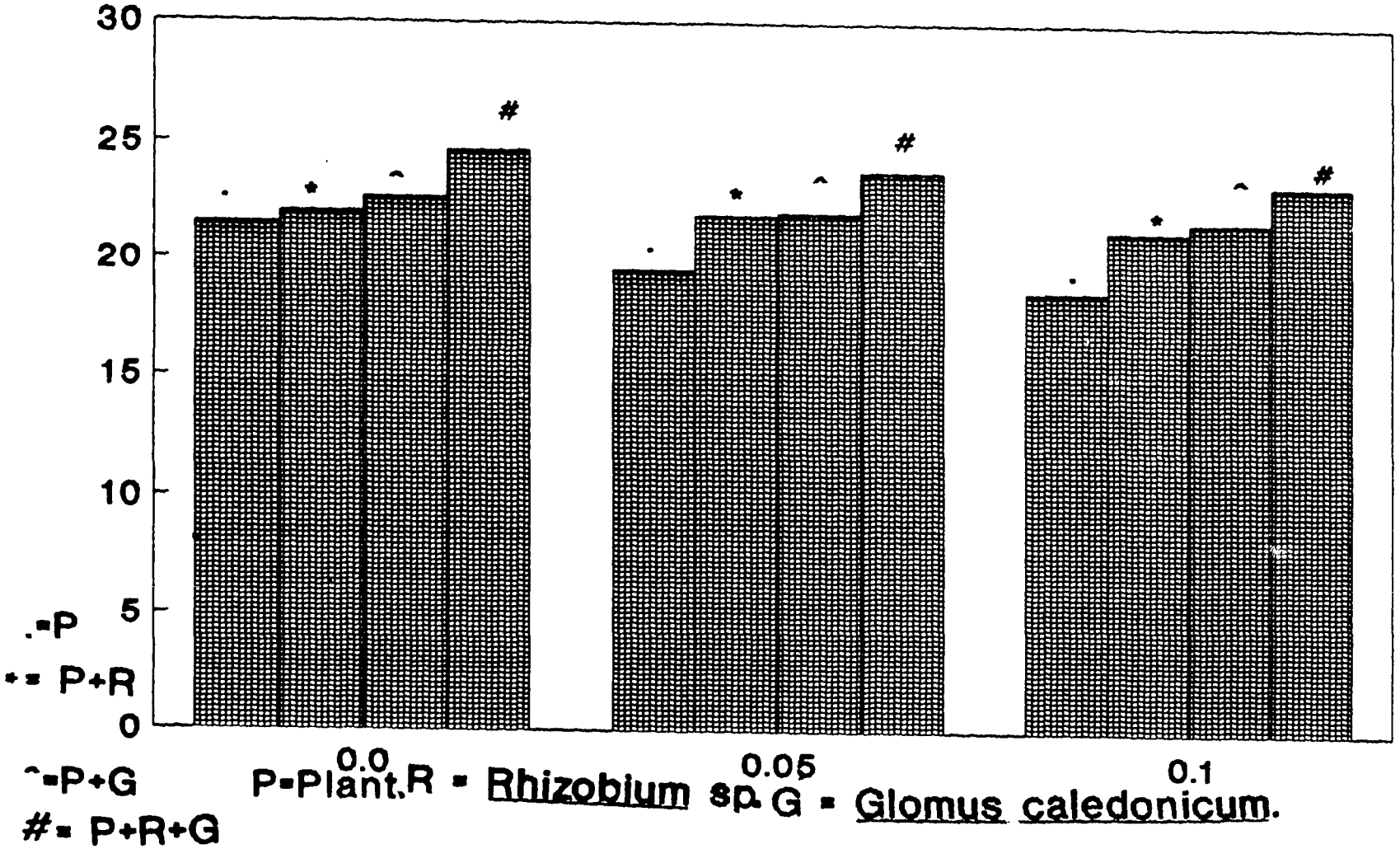

SO2 (ppm)
PROTEIN (%)
30
25
20
15
10
5
0
.=P
*= P+R
^=P+G
#= P+R+G
0.0
0.05
0.1
P=Plant.R = Rhizobium sp. G = Glomus caledonicum.

Fig.8

Root colonization,spore production and phosphorus content

Root colonization by the VAM fungus and and its spore production were greater in dual inoculated plants *(Rhizobium* sp. + *G.caledonicum*) than plants inoculated singly with the VAM fungus, *G.caledonicum.* Phosphorus content of dual inoculated plants was also greater than the single inoculated plants. Significant increase in phosphorus content occurred in single inoculated (with *G.caledonicum*) plants as compared to control (uninoculated). SO_2 at both the concentrations (0.05 and 0.1ppm) significantly suppressed root colonization and spore production by the VAM fungus. The reductions in root colonization was 2.8 and 5.7% in mycorrhizal plants and 2.3 and 2.5%in dual inoculated at 0.05 and 0.1 ppm SO_2,respectively, compared to their respective controls (unexposed inoculated plants)(Table 4; Fig.9; Plate II).Spore production by the VAM fungus was also suppressed at both concentrations of SO_2 in mycorrhizal as well as in dual inoculated plants. Spore production in single inoculated (mycorrhizal) plants was 46.8 and 65.4% less than control when exposed to the SO_2 concentrations. In dual inoculated plants, suppression in spore production was less than mycorrhizal plants. The reduction was 12.5 and 25% at 0.05 and 0.1 ppm SO_2 respectively.Therefore, in dual inoculated plants exposed to SO_2 concentrations, root colonization and spore production both were higher than exposed mycorrhizal plants.

Phosphorus content was also influenced by SO_2 exposures. Inoculations of root symbionts increased the phosphorus content of the plants. Shoot phosphorus in unexposed uninoculated plants was 60.2 , 54.1 and 6.29% less than dual ,mycorrhizal and nodulated unexposed plants. Inoculated plants (mycorrhizal, nodulated and dual inoculated) exposed to SO_2 concentration had significantly less phosphorus in shoots than unexposed inoculated plants (respective controls).Phosphorus content of roots of unexposed and uninoculated plants was also significantly less in comparison to mycorrhizal, nodulated and dual inoculated unexposed plants. Like shoot, mycorrhizal, nodulated and dual inoculated plants of exposed sets showed significantly less phosphorus in root than unexposed inoculated plants.Phosphorus content of root and shoot of dual inoculated exposed plants even after suppression by SO_2, was significantly greater than uninoculated (with either of the symbionts) and unexposed (to either concentrations of SO_2) plants. (Table 4; Fig.10).

Table 4. Effect of SO_2 on root colonization, spore number of VAM fungus *Glomus caledonicum* and phosphorus content of shoot and root of black gram plants inoculated with root symbionts.

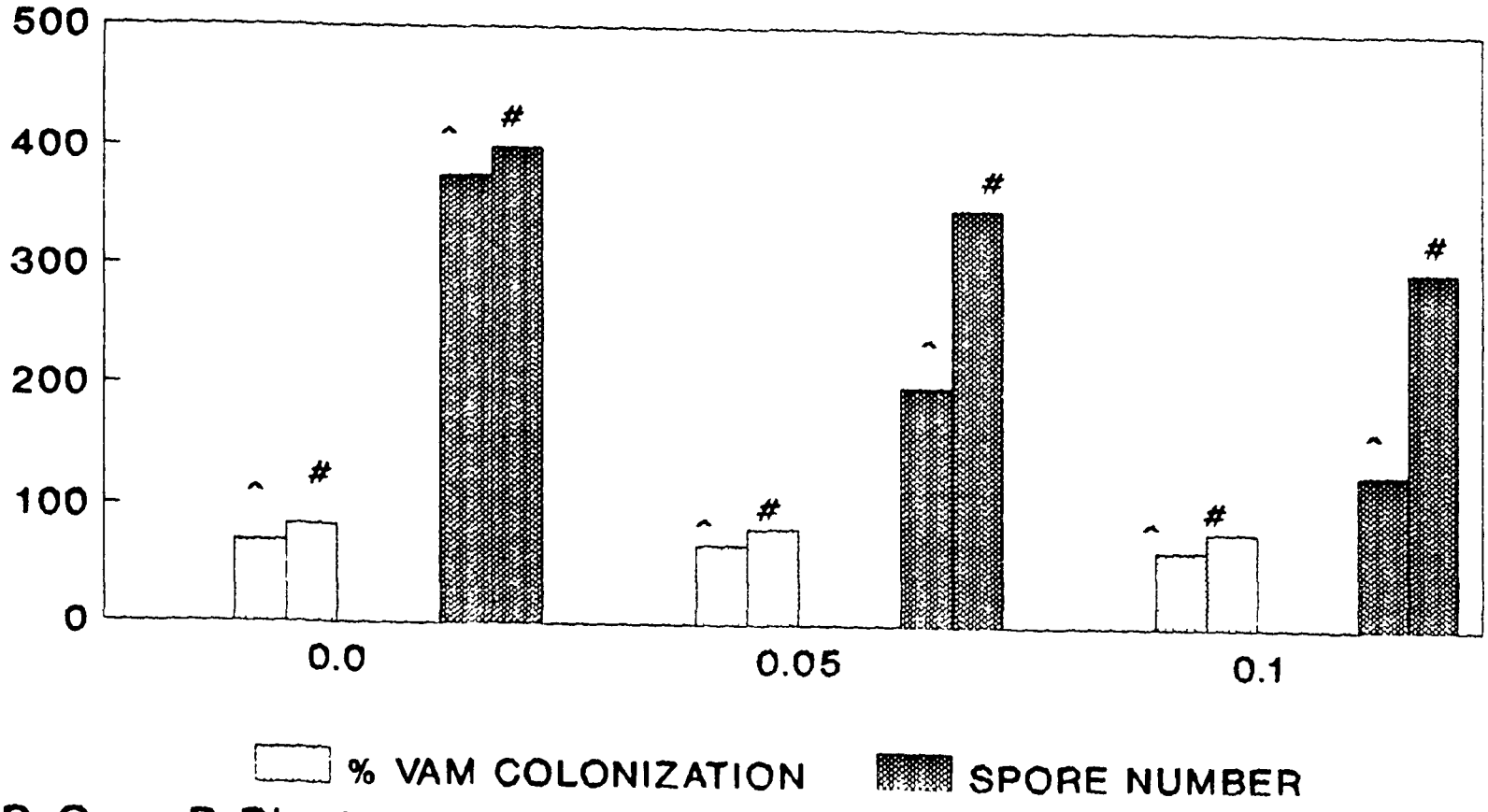
Treatment		SO_2(ppm)						
		Root colonization/Spore number				Phosphorus(%)		
		0.0	0.05	0.1		0.0	0.05	0.1
Plant (Black gram		-	-	-	S	0.134	0.116	0.102
without root					R	0.102	0.097	0.086
symbionts)								
Plant +		-	-	-	S	0.143	0.128	0.119.
Rhizobium sp.					R	0.143	0.114	0.105
Plant + *Glomus*	Co	70.0	68	66	S	0.292	0.264	0.237
caledonicum	CN	376	200	130	R	0.205	0.194	0.134
Plant +	Co.	84	82.0	81.91	S	0.337	0.318	0.300
Rhizobium sp. +	CN	400	350	300	R	0.228	0.204	0.197
G.caledonicum								

	C.D.(P=0.05)			
	Root colonization	Spore number	Phosphorus(%)	
	Co	C.No.	S	R
Treatment	0.98087	1.1443	0.00111	0.00105
SO_2	1.13262	1.3214	0.00129	0.00121
Interaction	1.9617	2.288	0.00223	0.00210

Co= % Root colonization, **C.No**. = Spore number, **S**=Shoot, **R**=Root.

Each value is mean of six replicates.

SO2 (ppm)
ROOT COLONIZATION AND SPORE NUMBER OF VAM FUNGUS



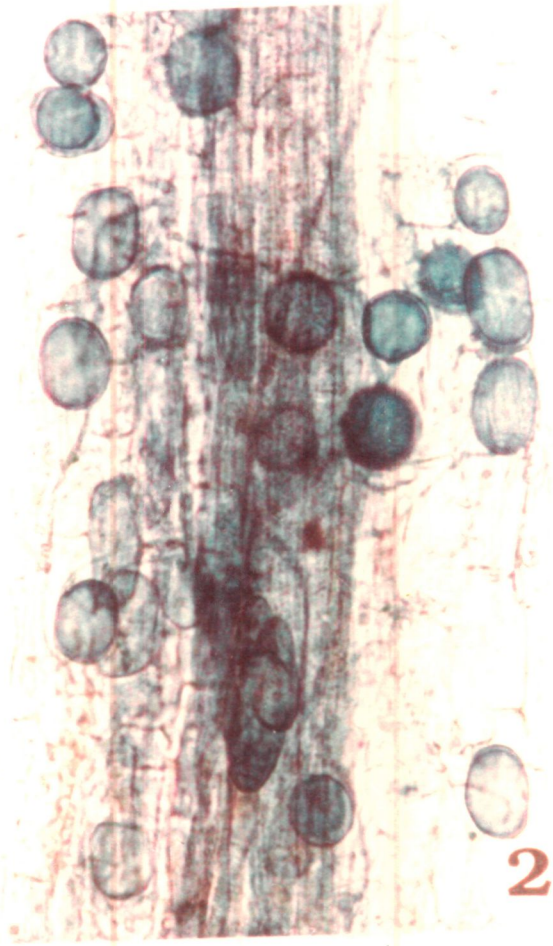
^=P+G P=Plant. R = Rhizobium sp. G = Glomus caledonicum.
#= P+R+G

Fig.9

Plate No. II :- Root colonization by *Glomus caledonicum* and its spores.

1. Arbuscles within the root cortex.
2. Vesicles within the root cortex.
3. Endophytic spores.
4. One enlarged spore.

1



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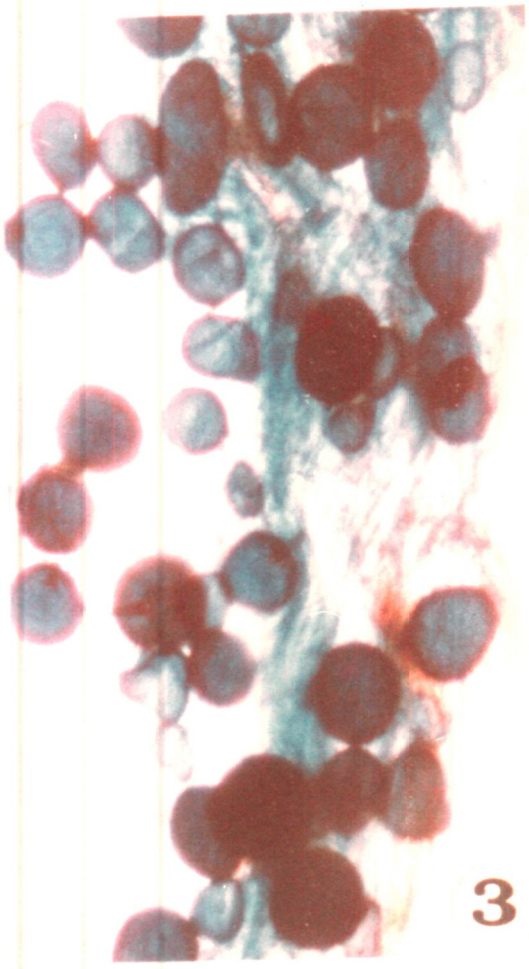

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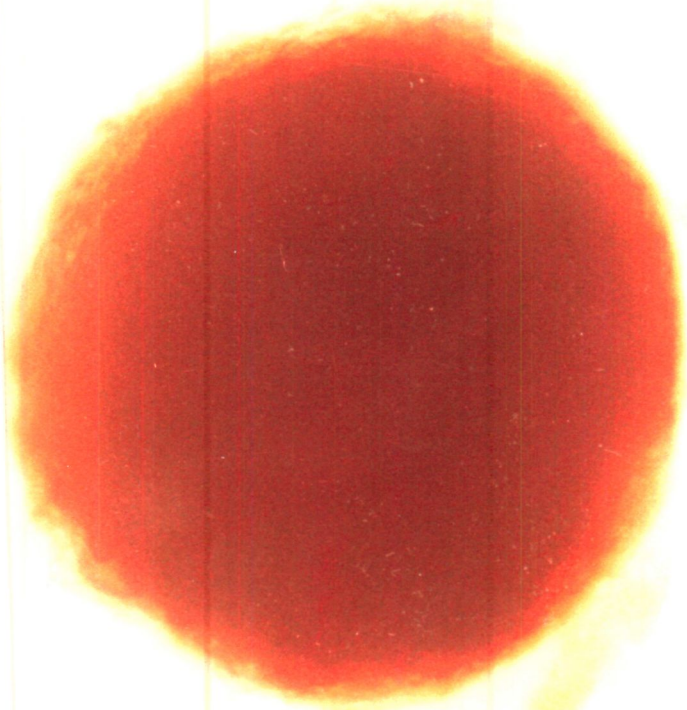

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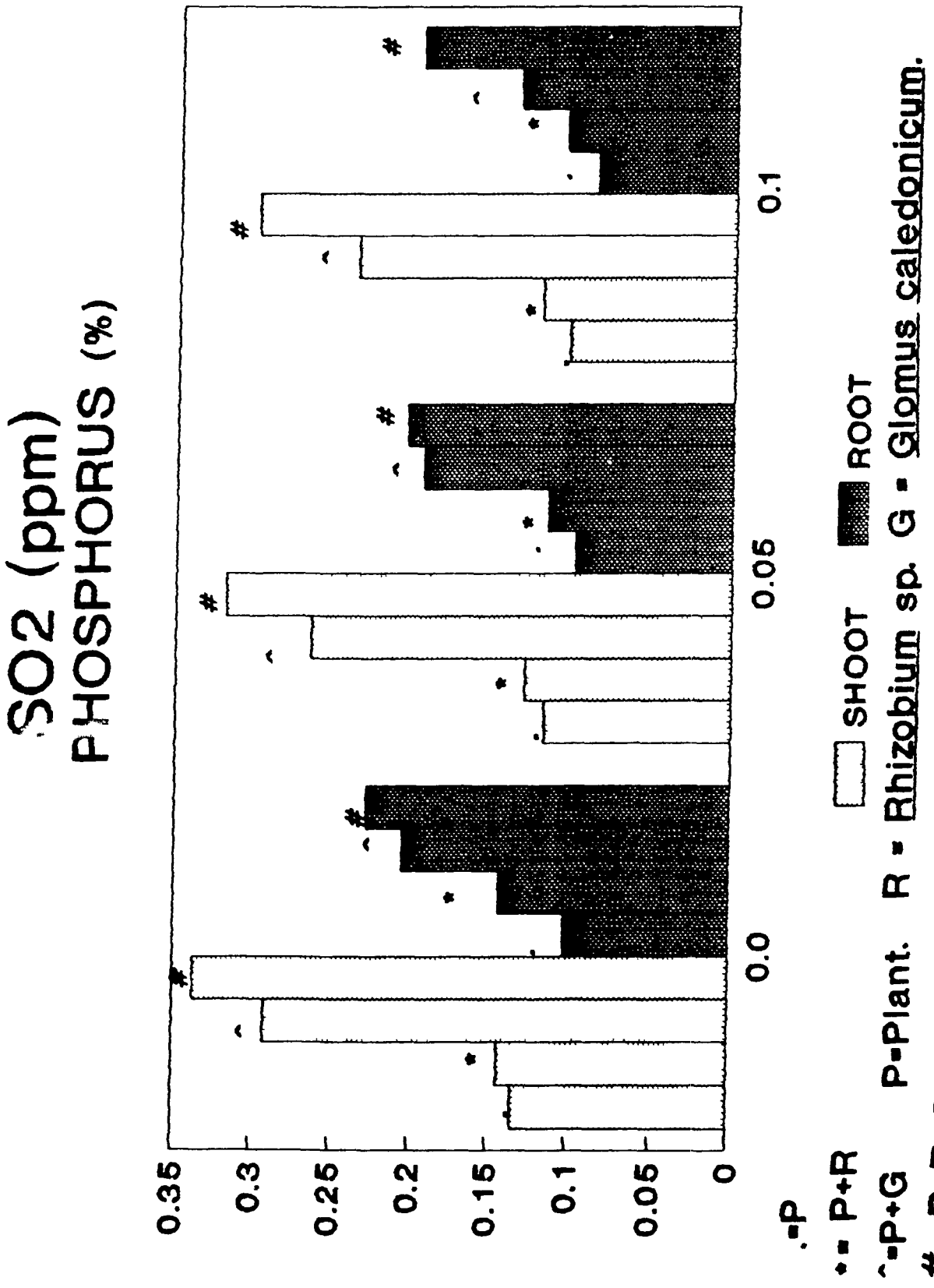

SO2 (ppm)
PHOSPHORUS (%)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.0
0.05
0.1
SHOOT
ROOT
.=P
*= P+R
^=P+G
#= P+R+G
P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.10

Root nodulation and nitrogen content

Root nodulation, determined by number of nodules per root system and their dry weight, was influenced by the various treatments. Influences also occurred on nitrogen content of the plants. Nodule number, dry weight of nodules and nitrogen content of the plant were significantly reduced in plants exposed to SO_2 concentrations, irrespective of the inoculation by the symbionts in comparison to their respective controls. Nodule number of nodulated unexposed plants was significantly small (27.7%) than unexposed dual inoculated plants. In dual inoculated plants, nodule number declined by 13.8 and 29.2% at 0.05 and 0.1 ppm SO_2 respectively compared to unexposed dual inoculated plants. In nodulated plants,exposures resulted in greater reduction in nodule number than dual inoculated exposed plants at both concentrations, being 14.9 and 31.9% less, respectively.Dry weight of nodules in nodulated unexposed plants was less by 1.9% when compared to unexposed dual inoculated plants. At both concentrations of SO_2,dual inoculated plants suffered 11.5 and 26.5% and nodulated plants 1.99 and 39.3% loss in the dry weight of nodules as compared to unexposed plants (Table 5; Fig.11).

Reduction in nitrogen content of shoot and root also occurred at both concentrations of SO_2 . Percent reduction in shoot nitrogen of uninoculated exposed plants was 14.9 and 24.7 at 0.05 and 0.1 ppm, respectively, compared to unexposed plants.Mycorrhizal, nodulated and dual inoculated plants of exposed sets showed a significant decline in nitrogen content of their shoots. Root nitrogen of uninoculated plants also significantly declined. Percent reduction in root nitrogen was 0.12 and 2.9 at both the concentration of SO_2, respectively, compared to unexposed plants. Shoot (26.4 and 23.5%)and root (70.1 and 58.9 %)of dual inoculated exposed plants had greater nitrogen content than unexposed uninoculated plants (Table 5;Fig.12).

OZONE

Plant growth

Like the experiment with SO_2, inoculation of black gram plants with the root symbionts resulted in enhancement of plant growth. Dual inoculation of the plants with the root nodule bacterium and the VAM fungus (*Rhizobium* sp.+ *G.caledonicum*) was more effective in increasing the plant growth than inoculation either with the VAM fungus,*G.caledonicum* and root nodule

Tabel 5. Effect of SO_2 on nodule number, nodule dry weight, nitrogen content of shoot and root of black gram plants inoculated with root symbionts.

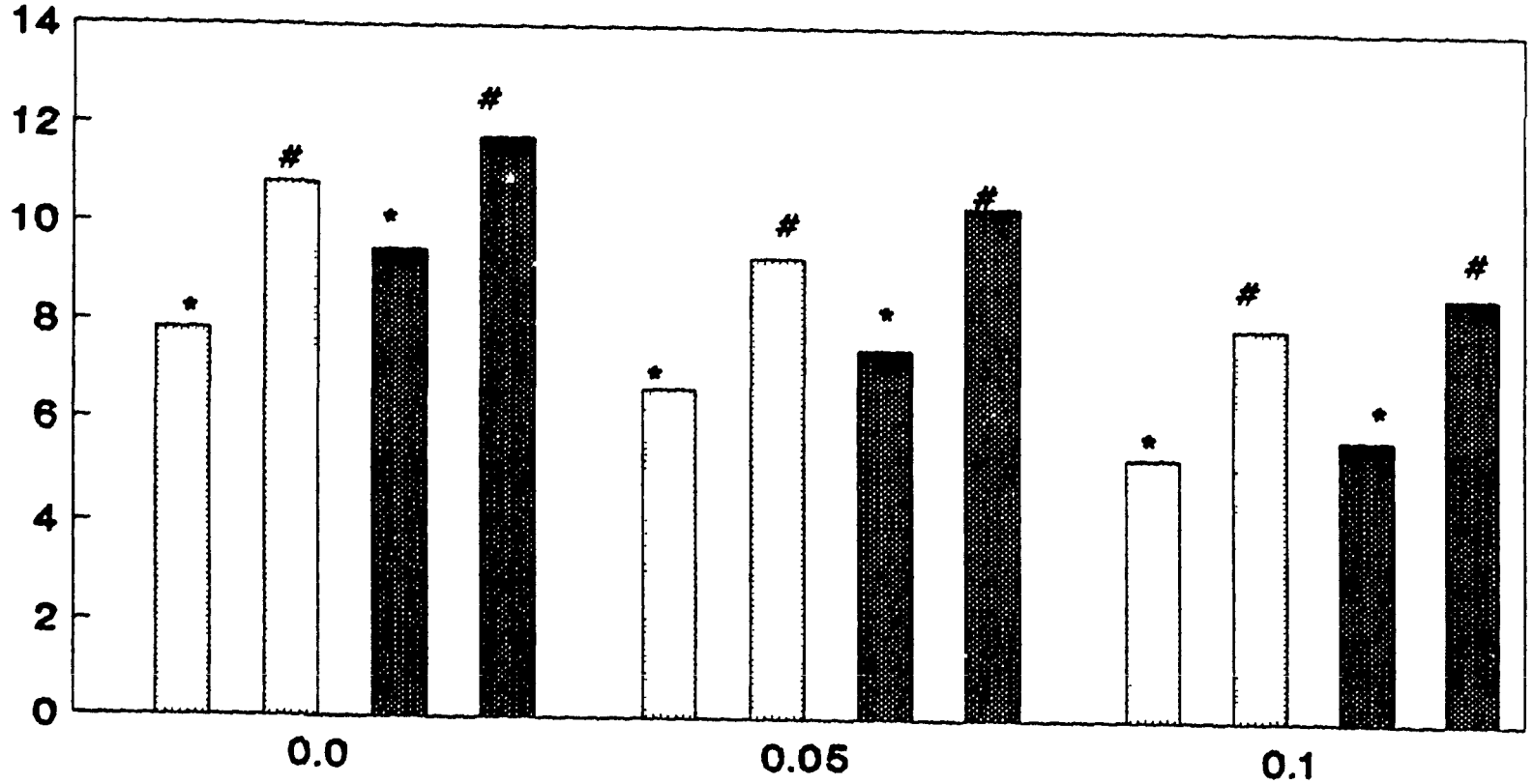
Treatment		SO_2 (ppm)						
		Nodule number/Nodule dry weight(mg)				Nitrogen (%)		
		0.0	0.05	0.1		0.0	0.05	0.1
Plant (Black gram					S	0.174	0.148	0.131
without root symbionts)		-	-	-	R	0.0804	0.0803	0.0780
Plant +	N.No	7.83	6.66	5.33	S	0.204	0.1910	0.174
Rhizobium sp.	D.W.	9.46	7.47	5.74	R	0.122	0.1190	0.1140
Plant +		-	-	-	S	0.215	0.208	0.196
Glomus					R	0.314	0.128	0.1190
caledonicum								
Plant +	N.No	10.83	9.33	7.96	S	0.238	0.220	0.215
Rhizobium sp. +	D.W.	11.75	10.39	8.63	R	0.149	0.136	0.127
G. *caledonicum*								

	C.D.(P=0.05)			
	Nodule number	Dry weight	Nitrogen (%)	
	N.No.	D.W.	S	R
Treatment	0.5851	0.01309	0.00915	0.001082
SO_2	0.6757	0.01511	0.01572	0.0012503
Interaction	1.1703	0.02618	0.018312	0.002165

N.No = Nodule number, **D.W.**= Dry weight of nodule, **S** = Shoot, **R** = Root.

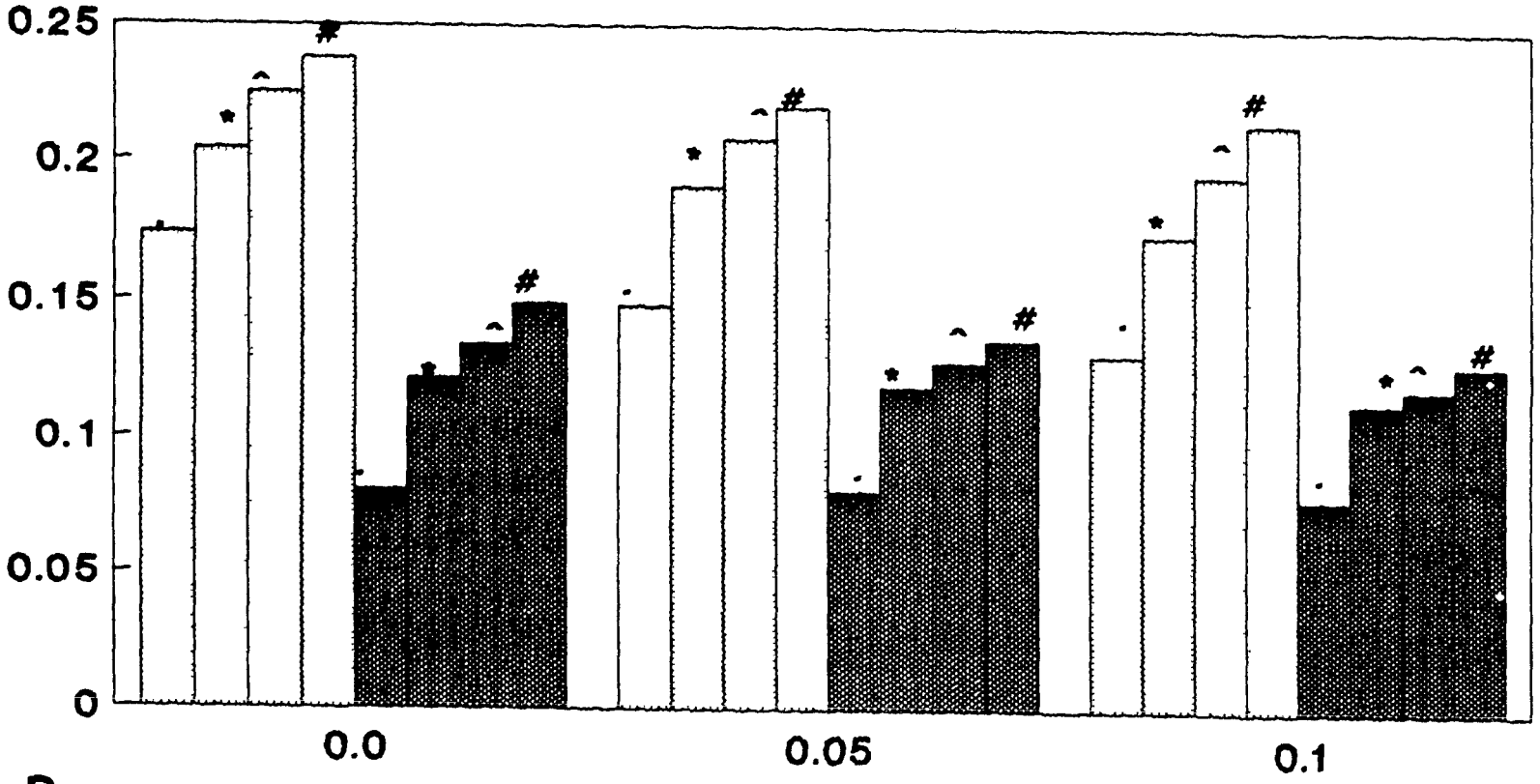
Each value is mean of six replicates.

SO2 (ppm)
ROOT NODULATION



*= P+R P=Plant. R = Rhizobium sp. G = Glomus caledonicum.
#= P+R+G

Fig.11



.=P

*= P+R

^=P+G

#= P+R+G

P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.12

bacterium, *Rhizobium* sp. All the determined plant growth parameters (length, fresh and dry weights of shoot and root) of dual inoculated plants measured greater than plants inoculated with *Rhizobium* sp. or *G.caledonicum.* The VAM fungus was more effective in improving the all considered plant growth parameters than the root nodule bacterium. Dual inoculated plants showed 16.8% greater shoot length, 71.7% greater root length, 35.2% greater shoot fresh weight and 74.2% greater root fresh weight, 13.5% greater shoot dry weight, 39.4% greater root dry weight than uninoculated plants.Plants of the treatments designated to receive O_3 exposures at 0.02-0.05-0.02 and 0.05-0.1-0.05 ppm according to the experimental design were treated in the chambers as described in Materials and Methods section. Both concentrations of O_3 caused plant growth suppressions. Significant reduction occurred in lengths of root and shoot at both concentrations of O_3 as compared to control (Table 6;Fig.13).

Reductions in shoot length at both concentrations of O_3 in uninoculated plants was 10.8 and 16.7%, in nodulated plants 8.92 and 25.6%, in mycorrhizal plants 3.3 and 15.4% and in dual inoculated plants 0.70% and 5.30%,respectively. Reduction in shoot length of dual inoculated plants was, however, not significant at 0.02-0.05-0.02 ppm of O_3. Root lengths of plants in all the treatments were also significantly reduced at both the concentrations. Uninoculated plants suffered greater plant growth loss with respect to shoot and root length than nodulated, mycorrhizal and dual inoculated plants. Among the two treatments of O_3, 0.05-0.1-0.05 ppm was more effective than the 0.02-0.05-0.02 ppm in suppressing the plant length (Table 6;Fig. 13).

Fresh weights of shoot and root were also significantly reduced at both treatments of O_3. Shoot fresh weight of nodulated, mycorrhizal and dual inoculated plants exposed to both O_3 treatment sequences were significantly ($P=0.05$) suppressed in comparison to their respective controls. Reduction was greater in nodulated plants than mycorrhizal plants. Reduction was lowest in dual inoculated plants at both O_3 treatment sequences. Even after reductions caused by O_3 treatments, fresh weights of root and shoot of dual inoculated and single inoculated plants were greater than uninoculated control plants (Table 6;Fig.14).

Like wise, both O_3 treatments sequences significantly reduced ($P=0.05$) dry weights of shoot and root. Reduction in shoot dry weight was greater in

Table 6. Effect of O_3 on length and fresh weight of shoot and root of black gram plants inoculated with root symbionts.

Treatment		O_3 (ppm)					
		Length (cm)			Fresh Weight (g)		
		0.0	0.02-0.05-0.02	0.05-0.1-0.05	0.0	0.02-0.0500.02	0.05-0.1-0.05
Plant (Black gram	S	36.02	32.10	28.33	9.75	6.8	4
without root	R	7.34	6.58	6.08	1.99	1.55	1.04
symbionts)							
Plant +	S	38.10	34.7	30	11.43	7.82	5.2
Rhizobium sp.	R	7.99	6.91	6.16	2.260	1.58	1.45
Plant + *Golmus*	S	38.80	37.50	32.80	11.7	8.88	7.15
caledonicum	R	8.41	7.52	7.12	3.320	2.64	2.15
Plant +	S	42.10	41.30	36.70	15.05	13.5	10.59
Rhizobium sp. +	R	13.6	11.75	11.10	7.720	7.04	5.75
G. caledonicum							

	C.D. (P=0.05)			
	Length		Fresh weight	
	S	R	S	R
Treatments	1.1006	0.87162	0.2580	0.01742
O_3	1.27089	1.0064	0.2979	0.02011
Interaction	2.2012	1.7432	0.51614	0.0348

S = Shoot, **R** = Root

Each value is mean of six replicates.

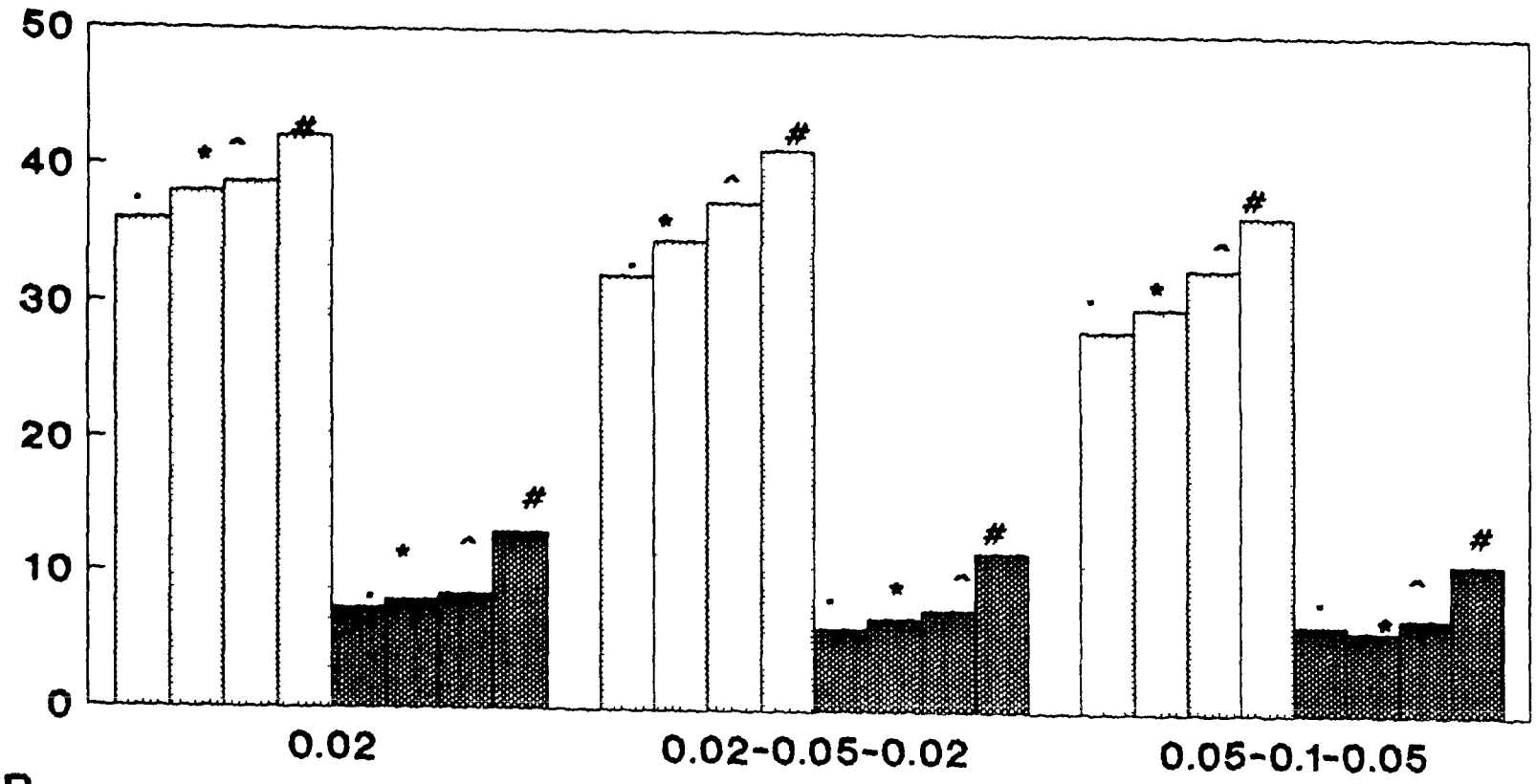
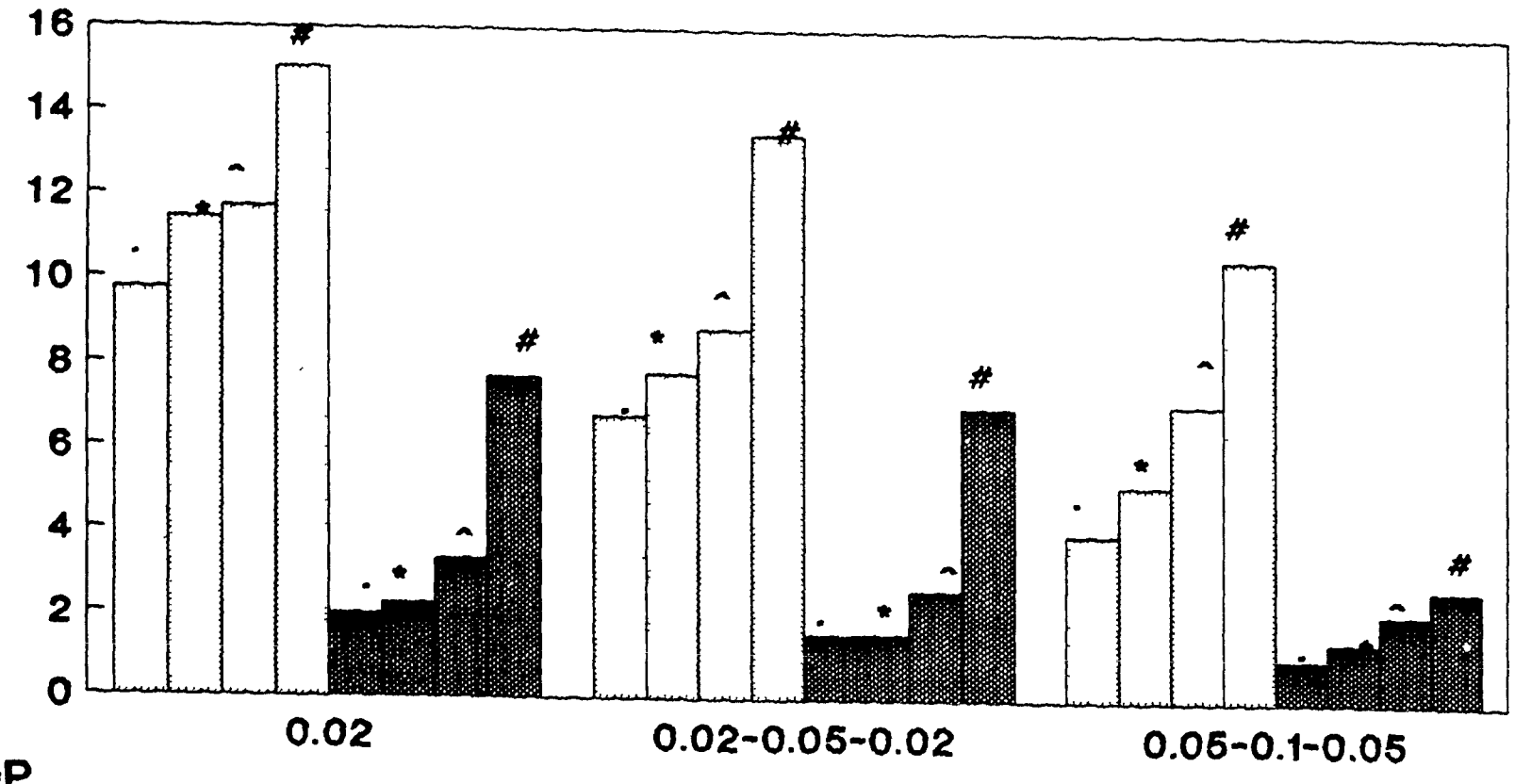

O3 (ppm)
LENGTH (cm)
50
40
30
20
10
0
0.02
0.02-0.05-0.02
0.05-0.1-0.05
.=P
*= P+R
^=P+G
#= P+R+G
SHOOT
ROOT
P=Plant. R = Rhizobium sp.G = Glomus caledonicum.

Fig.13



.=P

*= P+R

^=P+G

#= P+R+G

P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.14

uninoculated plants than, nodulated, mycorrhizal and dual inoculated plants in both treatments of O_3. Further, reduction was greater in nodulated plants than mycorrhizal plants. Reduction was least in dual inoculated plants. Similar trend in suppression of root dry weight caused by both treatment sequences of O_3 was found (Table 7;Fig.15).

Yield

Yield of black gram plants (pod number/plant and number of seed/pod) of dual inoculated plants (*Rhizobium* sp. + *G.caledonicum*), single inoculated plants either with *Rhizobium* sp.or with *G.caledonicum* was found to be greater than uninoculated plants. Dual inoculated unexposed plants showed 58.3% greater pod number than uninoculated unexposed (control) plants. Both O_3 treatment sequences suppressed pod number significantly. Uninoculated exposed plants showed 33.1 and 33.7% reductions in pod number at both the treatments, respectively, in comparison to uninoculated unexposed plants (control). Pod number of dual, mycorrhizal, nodulated exposed plants were also significantly reduced at both sequences, in comparison to their respective unexposed controls. Pod numbers of singly or dual inoculated exposed plants were still greater than uninoculated unexposed (control) plants. It was greatest in dual inoculated plants (Table 7;Fig .16).

Significant reduction in number of seeds occurred in both exposure treatments of O_3. Percentage decrease in number of seeds in uninoculated plants was 52.3 and 77.4, in nodulated plants 35.6 and 63.1, in mycorrhizal plants 33.7 and 53.6 and dual inoculated plants 28.9 and 42.3 respectively, in comparison to their respective unexposed (control) sets. Seed numbers of dual inoculated plants at both treatments of O_3, were though suppressed but the values were still greater than uninoculated unexposed plants (Table 7; Fig. 16).

Leaf chlorophyll and seed protein

Both sequences of O_3 concentrations adversely affected leaf chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll) and seed protein. Exposure of uninoculated plants to both sequences of O_3 concentrations resulted in a significant reduction in chlorophyll content of leaves in comparison to uninoculated unexposed plants. Dual inoculated, mycorrhizal and nodulated plants exposed to O_3 at both sequences also showed decline in

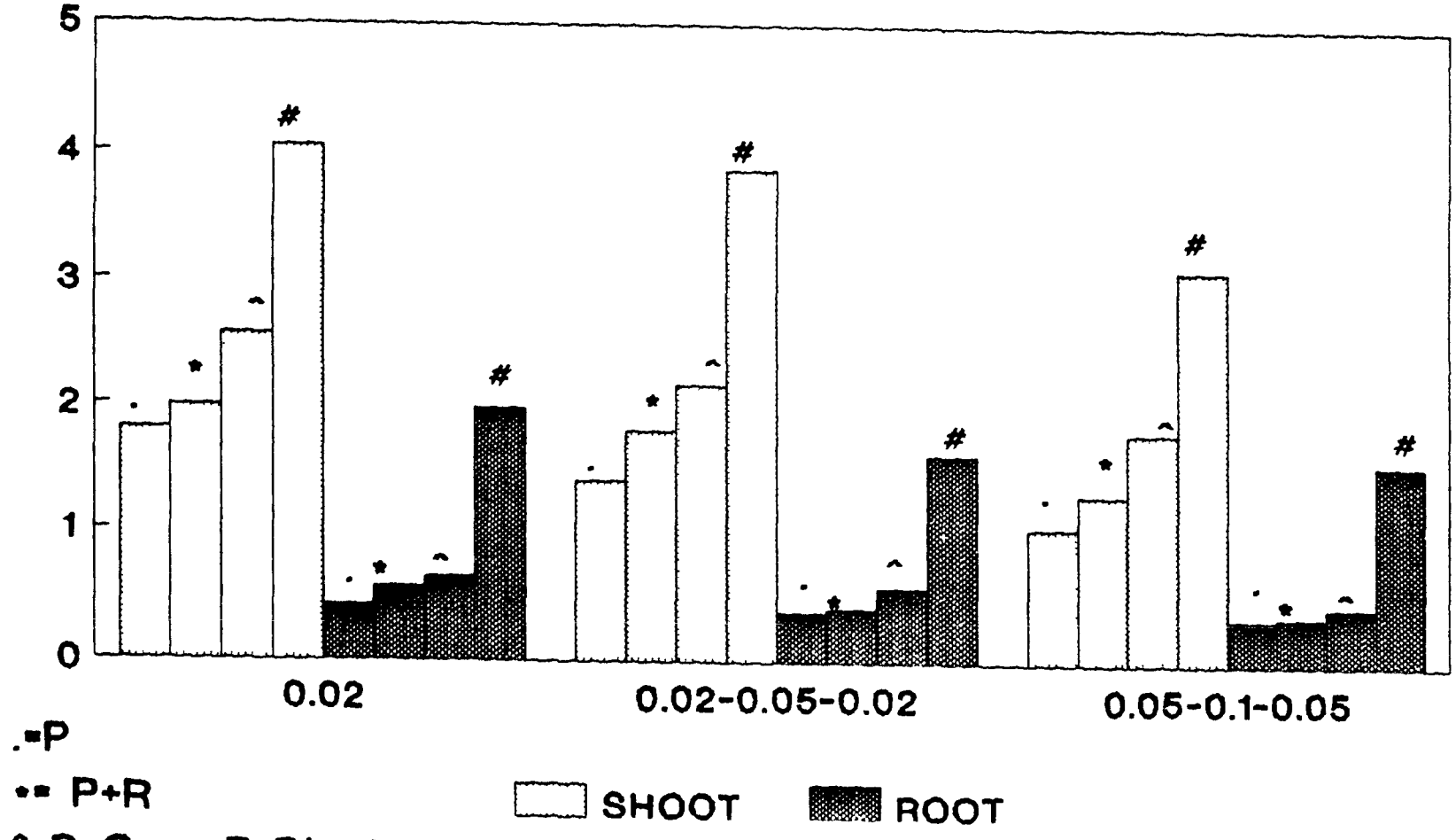
Table 7. Effect of O_3 on dry weight of shoot and root and yield of black gram plants inoculated with root symbionts.

Treatment		SO_2(ppm)						
		Dry weight (g)				Yield		
		0.0	0.02-0.05-0.02	0.05-0.1-0.05		0.0	0.02-0.05-0.02	0.05-0.0-0.05
Plant (Black gram without root symbionts)	S	1.80	1.40	1.04	P.No.	6.10	4.08	3.83
	R	0.421	0.374	0.344	S.No.	17	8.1	3.83
Plant + *Rhizobium* sp.	S	1.99	1.80	1.30	P.No.	6.80	6.08	4.50
	R	0.562	0.409	0.365	S.No.	24.4	15.7	9
Plant + *Golmus caledonicum*	S	2.57	2.167	1.80	P.No.	6.83	6.33	6.30
	R	0.644	0.569	0.440	S.No.	34.1	22.6	15.8
Plant + *Rhizobium* sp. + *G. caledonicum*	S	4.05	3.88	3.10	P.No.	9.66	7.66	7.60
	R	1.98	1.62	1.560	S.No.	67.1	47.7	38.5

	C.D. (P=0.05)			
	Dry Weight		Yield	
	S	R	P.No.	S.No.
Treatment	0.06023	0.00375	0.7448	2.011533
O_3	0.06954	0.00433	0.86003	2.32271
Interaction	0.1204	0.00751	1.4896	4.02306

S = Shoot, **R** = Root, **P.No**. =Pod number,, **S. No**.= Seed number

Each value is mean of six replicates.



P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.15

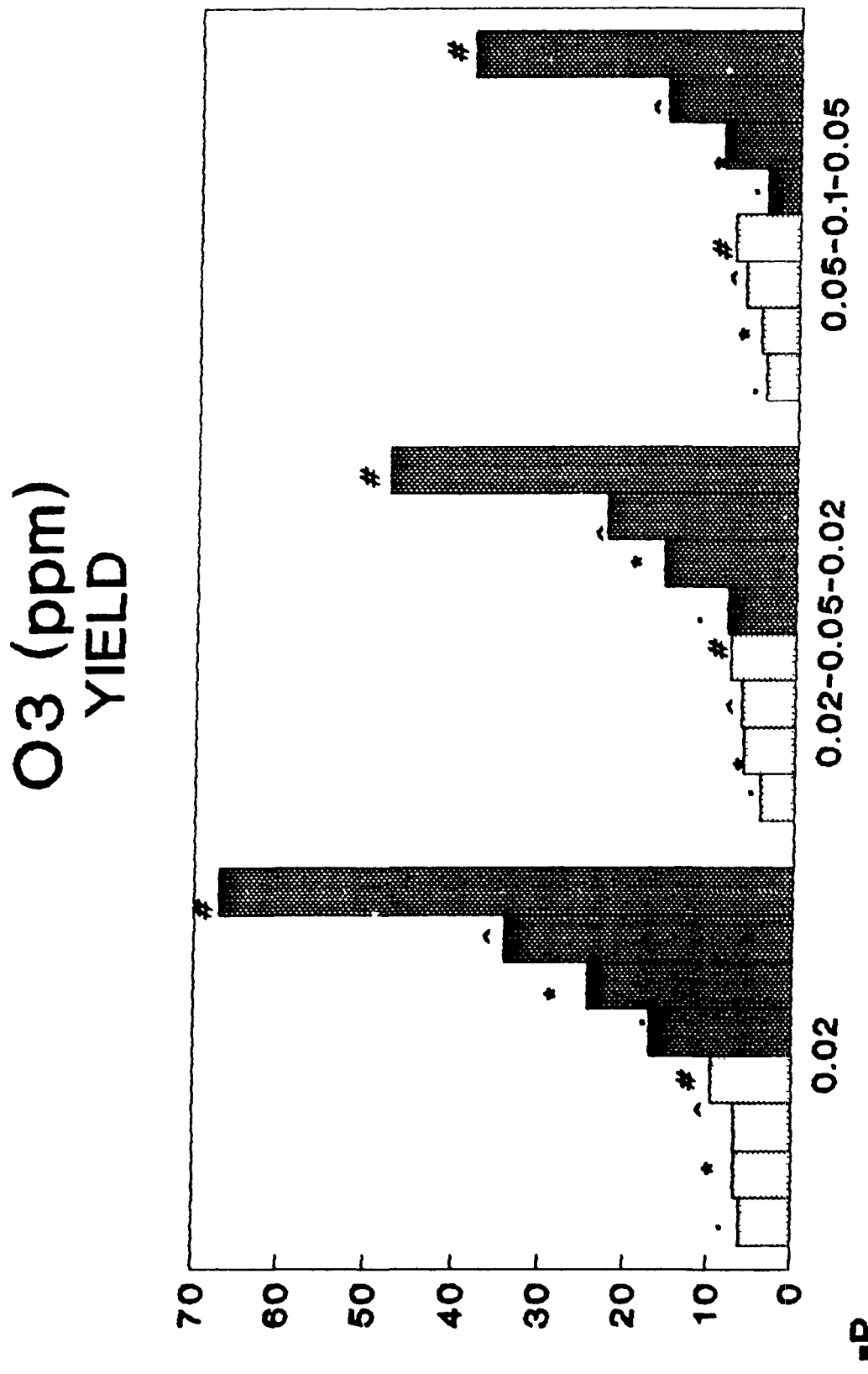

O3 (ppm)
YIELD
70
60
50
40
30
20
10
0
0.02
0.02-0.05-0.02
0.05-0.1-0.05
POD NUMBER
NUMBER OF SEEDS
.=P
*= P+R
^=P+G
#= P+R+G
P=Plant.R = Rhizobium sp. G = Glomus caledonicum.

Fig.16

Table 8. Effects of O_3 on chlorophyll contents of leaf and seed protiein of black gram plants inoculated with root symbionts.

Treatment		O_3 (ppm)					
		Chlorophyll (mg/g)			Protien(%)		
		0.0	0.02-0.05-0.02	0.05-0.1-0.05	0.0	0.02-0.05-0.02	0.05 0.0-0.05
Plant (Black gram without root symbionts)	a	0.621	0.525	0.414			
	b	0.320	0.300	0.200	21.66	18.31	17.08
	T	0.975	0.900	0.620			
Plant+ *Rhizobium* sp.	a	0.647	0.642	0.620			
	b	0.469	0.344	0.322	22.16	19.83	18.65
	T	1.260	1.205	1.012			
Plant + *Golmus caledonicum*	a	0.681	0.669	0.622			
	b	0.473	0.399	0.362	22.83	20.80	22.01
	T	1.289	1.287	1.208			
Plant+ *Rhizobium* sp.+ *G. caledonicum*	a	0.990	0.832	0.736			
	b	0.786	0.776	0.700	24.79	22.67	22.01
	T	2.00	1.452	0.259			

	C.D. (P=0.05)			
	Chlorophyll			Protein
	a	b	T	
Treatment	0.00344	0.00117	0.1151	0.01082
O_3	0.00397	0.00136	0.1329	0.012500
Interaction	0.00688	0.00235	0.2302	0.021651

a=Chlorophyll a , **b**=Chlorophyll b, **T**=Total Chlorophyll

Each value is mean of six replicates.

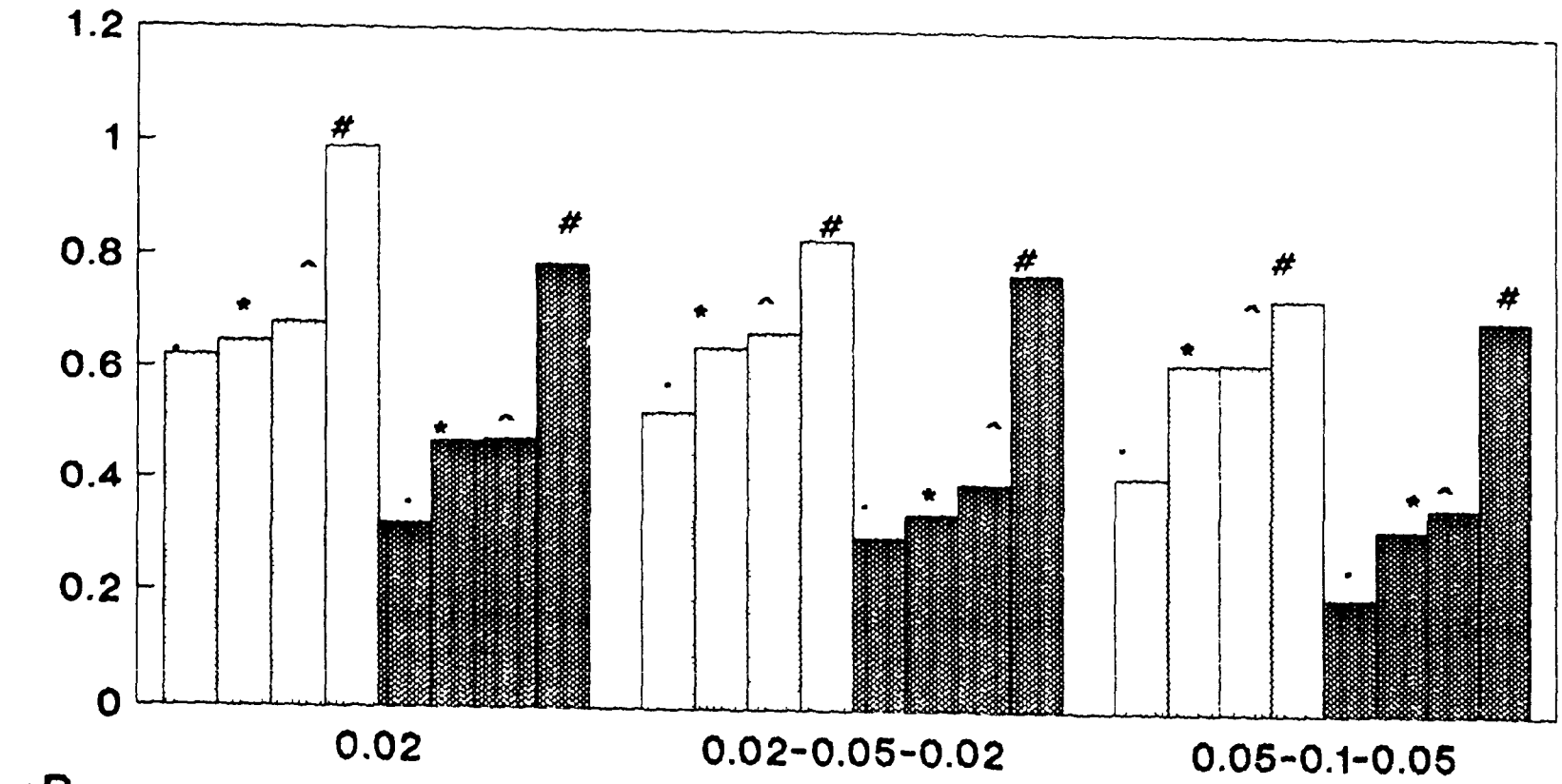

O3 (ppm)
CHLOROPHYLL (a,b) (mg/g)
1.2
1
0.8
0.6
0.4
0.2
0
0.02
0.02-0.05-0.02
0.05-0.1-0.05
.=P
*= P+R
^=P+G
#= P+R+G
CHLOROPHYLL a
CHLOROPHYLL b
P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.17

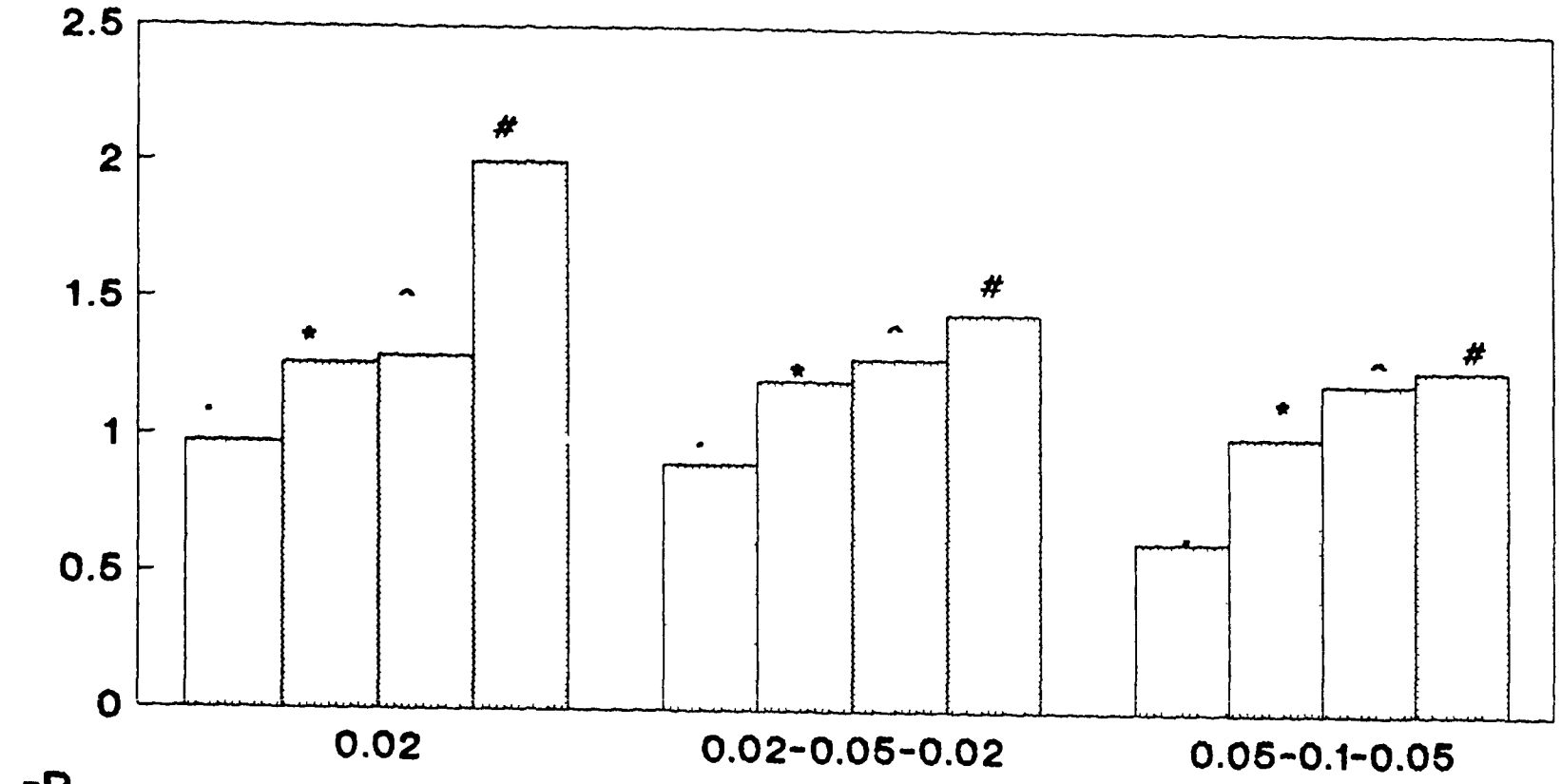

O3 (ppm)
TOTAL CHLOROPHYLL (mg/g)
2.5
2
1.5
1
0.5
0
0.02
0.02-0.05-0.02
0.05-0.1-0.05
.=P
*= P+R
^=P+G
#= P+R+G
TOTAL CHLOROPHYLL
P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.18

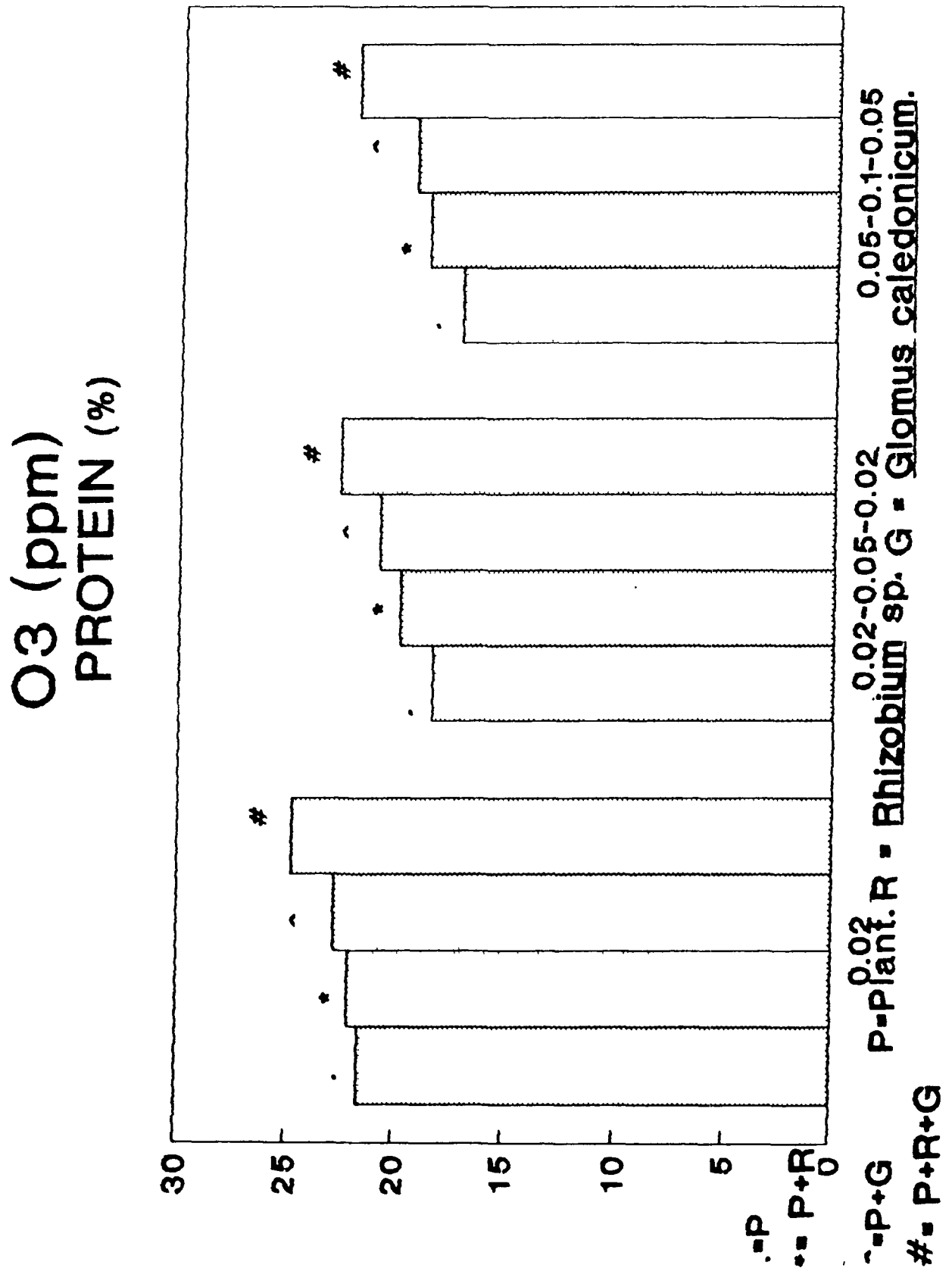

O3 (ppm)
PROTEIN (%)
30
25
20
15
10
5
0
.=P
*= P+R
`=P+G
#= P+R+G
P=Plant. R = Rhizobium sp. G = Glomus caledonicum.
0.02
0.02-0.05-0.02
0.05-0.1-0.05

Fig.19

Table 9. Effect of O_3 on root colonization and spore number of VAM fungus *Glomus caledonicum* and phosphorus content of shoot and root of black gram plants inoculated with root symbionts.

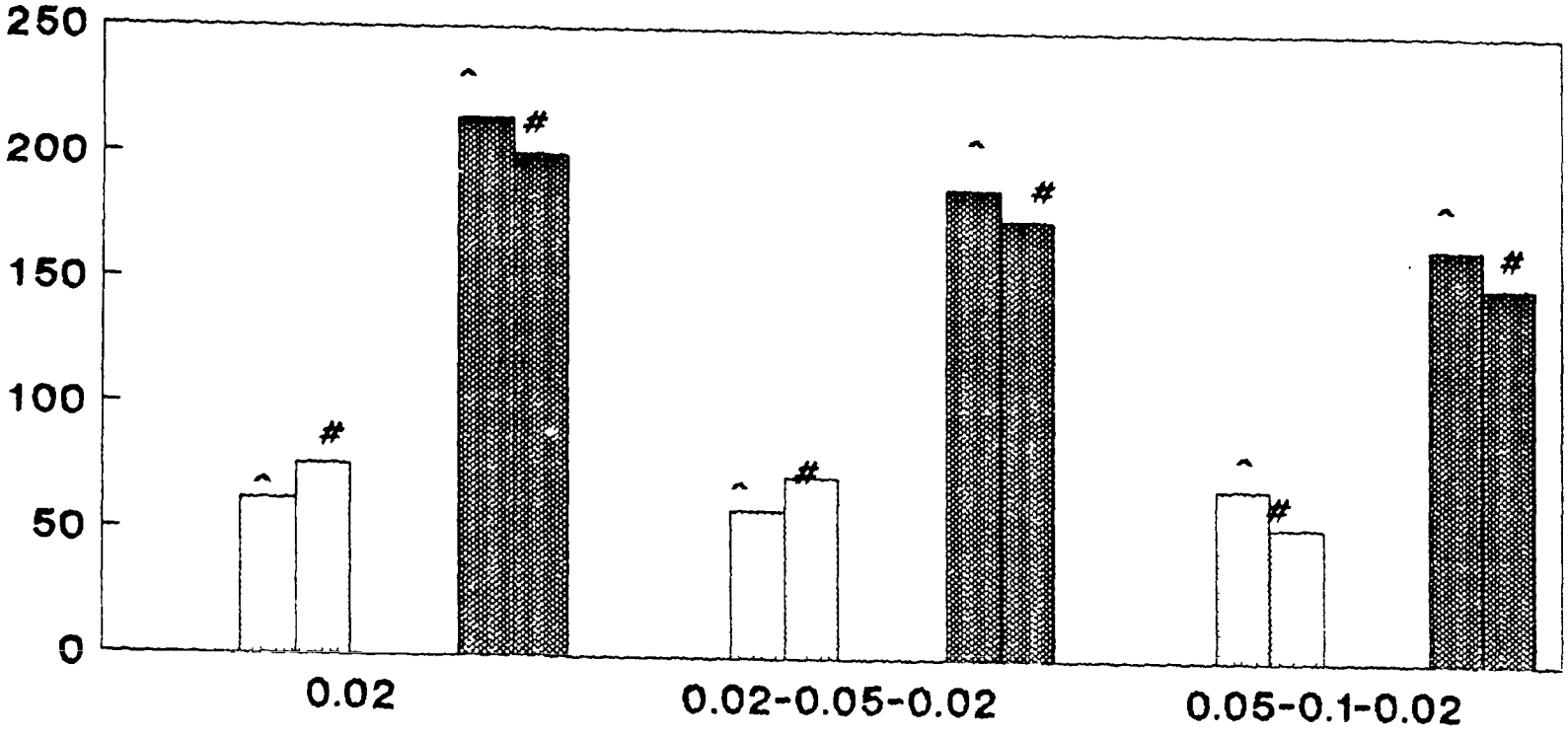
Treatment		O_3 (ppm)						
		Root colonization/Spore number				Phosphorus(%)		
		0.0	0.02-0.05-0.02	0.05-0.1-0.05		0.0	0.02-0.05-0.02	0.05 0.0-0.05
Plant (Black gram without root symbionts)		-	-	-	S	0.635	0.530	0.457
					R	0.064	0.041	0.036
Plant+ *Rhizobium* sp.		-	-	-	S	0.675	0.627	0.618
					R	0.067	0.049	0.044
Plant + *Golmus caledonicum*	Co	62.16	58.83	53.50	S	0.980	0.914	0.705
	C.No	200	175.0	150.0	R	0.086	0.068	0.60
Plant + *Rhizobium* sp. + *G. caledonicum*	Co.	76.16	72.16	68.66	S	1.255	1.223	1.184
	C.No	214.6	187.33	165.0	R	0.114	0.096	0.089

	C.D.(P=0.05)			
	Root Colonization	Spore number	Phosphorus(%)	
	Co	C.No.	S	R
Treatment	0.06006	0.826805	0.02298	0.001166
O_3	0.6935	0.95471	0.2654	0.001347
Interaction	1.2013	1.65361	0.4596	0.00233

Co =Root Colonization, **C.No**.= Spore number, **S**= Shoot, **R**= Root.

Each value is mean of six replicates

O3 (ppm)
ROOT COLONIZATION AND SPORE NUMBER OF VAM FUNGUS



^=P+G P=Plant. R = Rhizobium sp. G = Glomus caledonicum.
#= P+R+G

Fig.20

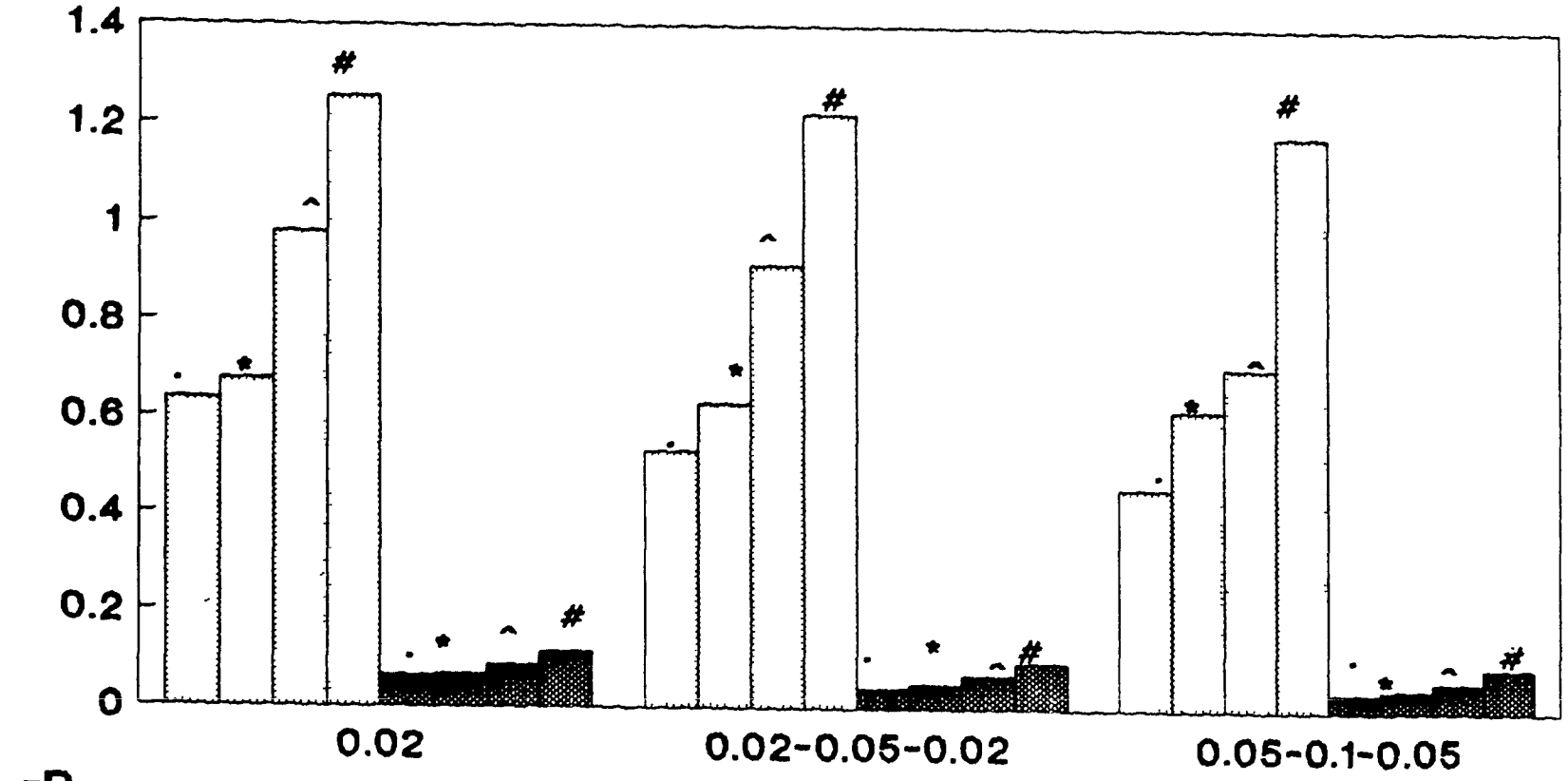

O3 (ppm)
PHOSPHORUS (%)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.02
0.02-0.05-0.02
0.05-0.1-0.05
.=P
*= P+R
^=P+G
#= P+R+G
SHOOT
ROOT
P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.21

phosphorus content of the plants at both 0.02-0.05-0.02 and 0.05-0.1-0.05 ppm of O_3. Percent reductions in phosphorus contents of shoot in dual inoculated plants were 2.5 and 5.6%, in mycorrhizal plants 67.3 and 28.06%, in nodulated plants 7.1 and 8.1% and uninoculated exposed plants 16.5 and 28% at both treatments of O_3 in comparison to their respective controls. Root phosphorus of dual inoculated, mycorrhizal, nodulated and uninoculated plants was reduced significantly at both concentrations of O_3, compared with their respective controls. Phosphorus contents of shoot and root of dual inoculated plants exposed to both concentrations of O_3 were significantly greater than uninoculated unexposed control plants (Table 9; Fig. 21).

Root nodulation and nitrogen content

Root nodulation and nitrogen content of black gram plants were enhanced by inoculation of the plants with root symbionts. Root nodulation was greater in plants inoculated with both *Rhizobium* sp. and *G.caledonicum* than plants inoculated with *Rhizobium* sp. alone. Similarly nitrogen was greater in dual inoculated plants than single inoculated plants. O_3 treatments suppressed root nodulation. At both concentrations, (0.02-0.05-0.02 and 0.05-0.1- 0.05 ppm) significant reductions occurred in nodule number, dry weight of nodules and nitrogen content of shoot and root.Nodule number was greater by 14.2% and dry weight of nodules by 25% in dual inoculated unexposed plants than nodulated unexposed plants. Nodule number and dry weight of nodules of dual inoculated and nodulated plants were significantly reduced by O_3 treatments in comparison to their respective controls (Table 10; Fig. 22).

Significant reduction occurred in nitrogen contents of shoot and root at both concentrations of O_3. Nitrogen content of shoot of dual inoculated exposed plants at both concentrations of O_3 showed a reduction of 17.6 and 18.2% respectively , in comparison to dual inoculated unexposed plants. Uninoculated , nodulated, mycorrhizal plants exposed to 0.02-0.05-0.02 and 0.05-0.1-0.05 ppm O_3 showed significant reduction of shoot and root nitrogen in comparison to their respective controls. Dual inoculated plants exposed to both concentrations of O_3 contained significantly greater root and shoot nitrogen than uninoculated control plants (Table 10; Fig.23).

chlorophyll contents of leaves compared to their respective controls. Chlorophyll contents of exposed dual inoculated or mycorrhizal and nodulated plants were greater (P=0.05) than uninoculated unexposed plants. Dual inoculated exposed plants contained chlorophyll a, 33.9 and 15.6%, chlorophyll b 58.7 and 54.2% and total chlorophyll 48.9 and 29.1% greater than uninoculated unexposed plants at two respective O_3 concentrations sequences (Table 8; Fig. 17 and 18).

Seed protein of mycorrhizal and dual inoculated plants exposed at both treatments of O_3 showed a significant reduction (P=0.05), in comparison to their respective controls. Dual inoculated, mycorrhizal and nodulated exposed plants had greater seed protein than uninoculated unexposed plants. Dual inoculated unexposed plants were 14.4% greater in seed protein than uninoculated unexposed plants (Table 8; Fig.19).

Root colonization , spore production and phosphorus content

Root colonization, spore production of the VAM fungus on black gram plants were greater in dual inoculated plants *(Rhizobium* sp. + *G.caledonicum)* than plants inoculated singly with *G.caledonicum*.Significant reductions occurred at 0.02-0.05 and 0.05-0.1-0.05 ppm O_3 .Root colonization of dual inoculated unexposed plants was greater by 25..2% than mycorrhizal unexposed plants. O_3 exposures at both the concentrations decreased root colonization. Reduction in dual inoculated plants was 5.2 and 9.8% and in mycorrhizal plants 5.3 and 13.9% at both concentrations of O_3, respectively, in comparison to their respective controls.

Spore number in soil grown with dual inoculated plants was greater than spore number in soil grown with plants with *G.caledonicum* alone.Spore number/100g soil of dual inoculated unexposed plants was greater (by 7.3%) than mycorrhizal unexposed plants. Both treatments of O_3, significantly reduced spore numbers. The reduction in dual inoculated plants was 12.7 and 23.1% and in mycorrhizal plants 12.5 and 25%, compared to their respective controls. Root colonization and spore production of dual inoculated exposed plants at both treatments of O_3 were also significantly greater than mycorrhizal unexposed plants (Table 9; Fig.20)

Shoot and root phosphorus content was also significantly influenced by O_3 treatments. Significant reduction (P=0.05) occurred in shoot and root

Table 10 Effect of O_3 on nodule number, nodule dry weight, nitrogen content of shoot and root of black gram plants inoculated with root symbionts

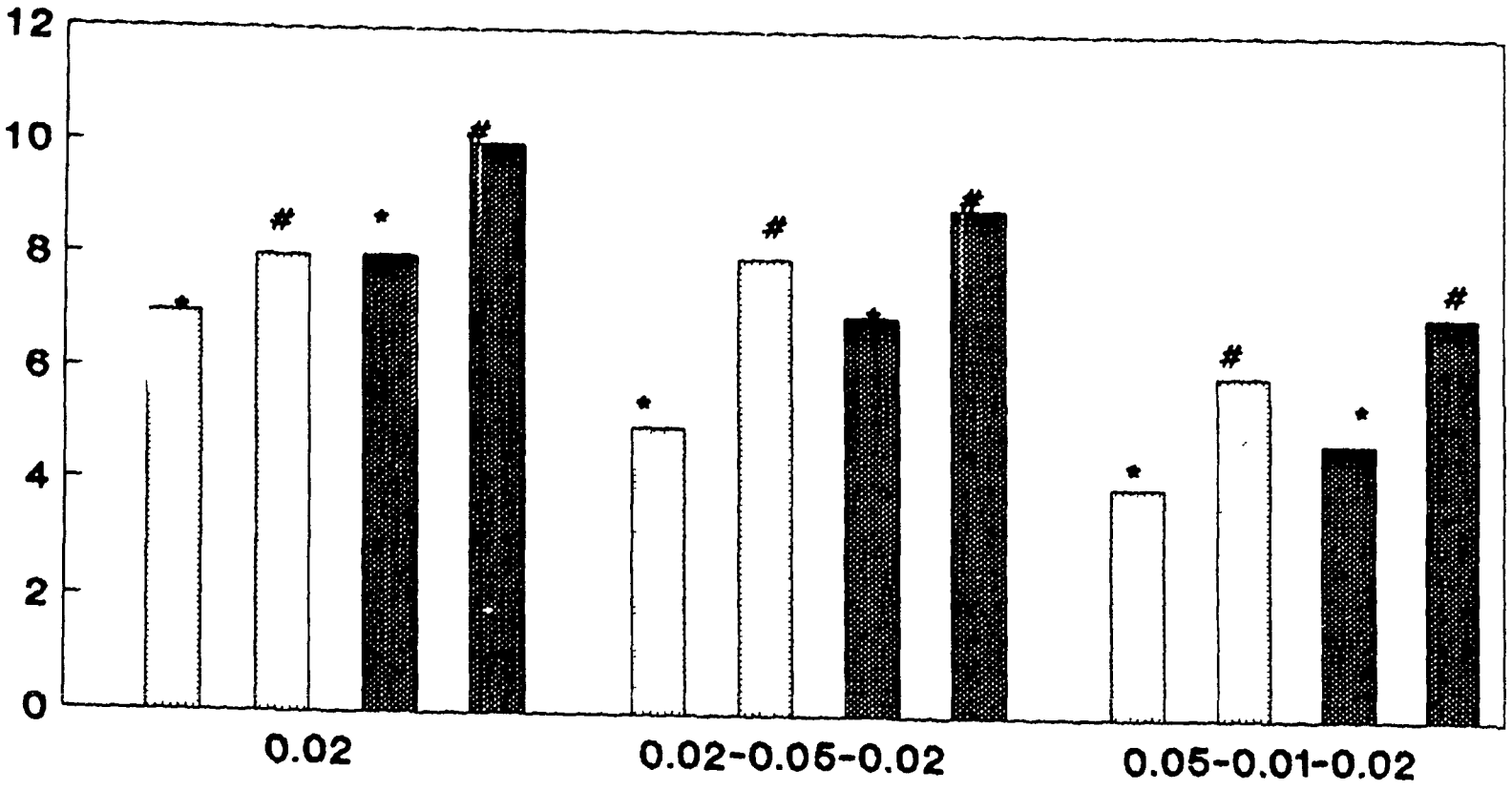
Treatment		O_3 (ppm)						
		Nodule number/Nodule dry weight(mg)				Nitrogen (%)		
		0.0	0.02-0.05-0.02	0.05-0.1-0.02		0.0	0.02-0.05-0.02	0.05 0.1-0.05
Plant (Black gram without root symbionts)		-	-	-	S	0.990	0.685	0.525
					R	0.200	0.174	0.166
Plant + *Rhizobium* sp.	N.No	7.0	5.0	4.0	S	1.315	1.038	1.015
	D.W.	8.0	7.0	4.8	R	0.252	0.235	0.231
Plant + *Golmus caledonicum*		-	-	-	S	1.584	1.139	1.026
					R	0.437	0.323	0.301
Plant + *Rhizobium* sp. + *G. caledonicum*	N.No	8.0	6.0	5.6	S	2.597	2.138	2.124
	D.W.	10	8.92	7.06	R	0.810	0.545	0.528

	C.D. (P=0.05)			
	Nodule number	Dry weight	Nitrogen (%)	
	N.No.	D.W.	S	R
Treatment	0.0655	0.59278	0.0238	0.00556
O_3	0.768	0.6844	0.02749	0.00642
Interaction	1.331	1.1855	0.04763	0.011129

N.No = Nodule number, **D.W.**= Dry weight of nodule, **S** = Shoot, **R** = Root.

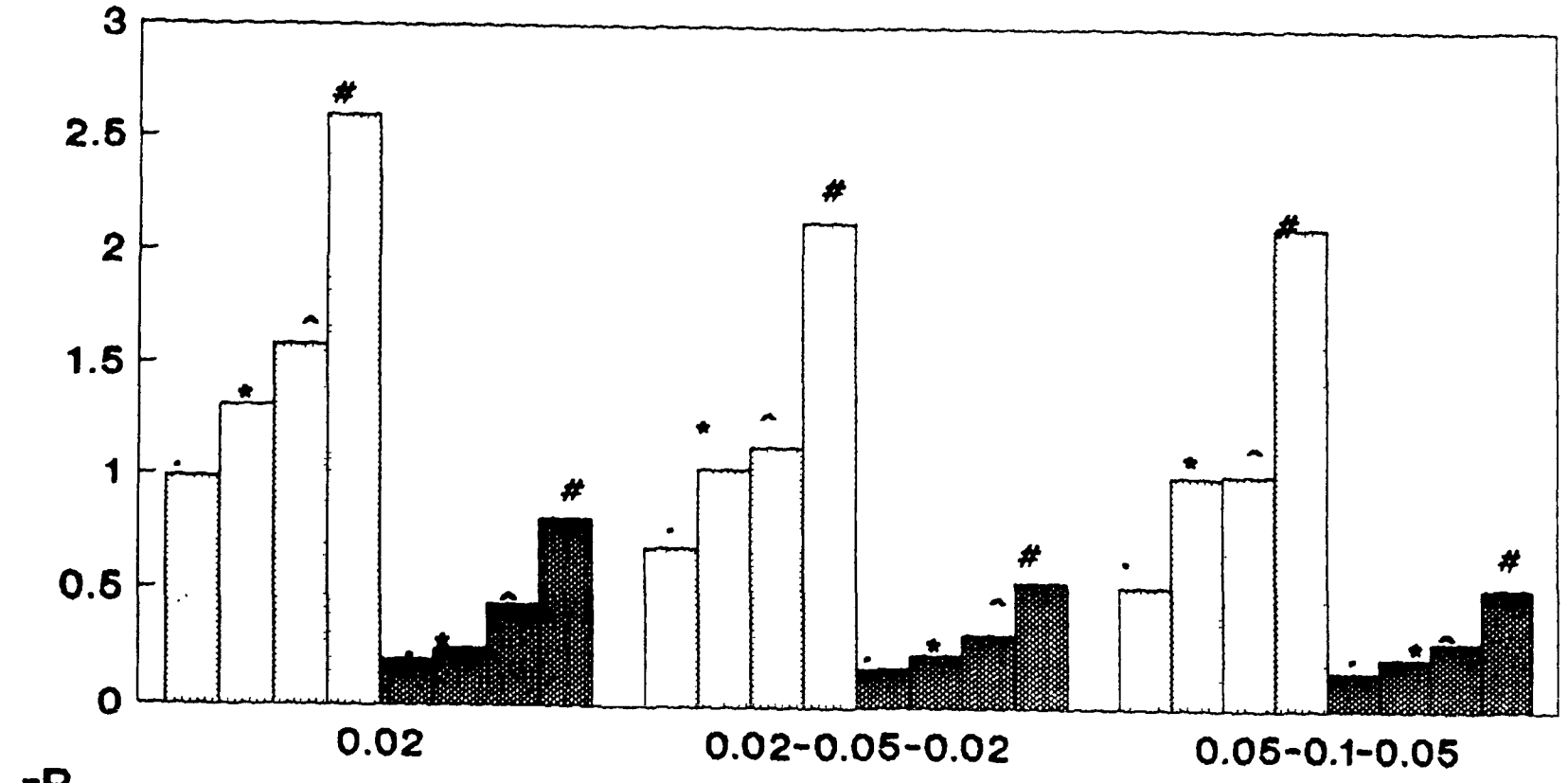
Each value is mean of six replicates.

O3 (ppm)
ROOT NODULATION



*= P+R
#= P+R+G
P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.22



.=P

*= P+R

^=P+G

#= P+R+G

P=Plant.R = Rhizobium sp. G = Glomus caledonicum.

Fig.23

FLY ASH

Plant growth

Like the experiments with SO_2 and O_3, plants of black gram showed an improved growth (length and fresh and dry weights of root and shoot) as a result of inoculation with the root symbionts, *Rhizobium* sp.and *G.caledonicum*. All the considered plant growth parameters were greater in dual inoculated plants (*Rhizobium* sp.+ *G.caledonicum*) than plants inoculated singly either with *Rhizobium* sp. or *G.caledonicum*.

Fly ash added in soil influenced plant growth. This effect was dependent on the level of fly ash applied to soil. Improvement in plant growth occurred upto 60% (i.e. 20,40,60%) levels of fly ash. Shoot and root lengths of inoculated and uninoculated plants showed significant increases in comparison to control plants grown in soil without fly ash amendment.

Shoots of uninoculated and nodulated plants showed improved length at 20,40 and 60% levels of fly ash but in mycorrhizal and dual inoculated plants no significant increase occurred at 20 and 40% levels. At 60% level,their lengths declined. Root length in uninoculated, nodulated and mycorrhizal plants showed a significant increase as compared to their respective controls upto 60% level. In dual inoculated plants, this increase was only upto 20%, with a decline at 40% onwards (Table 11; Fig. 24).

Fresh weights of shoot and root of nodulated, mycorrhizal and dual inoculated plants were significantly (P=0.05) enhanced in comparison to uninoculated plants. Significant increases (P=0.05) in fresh weights of shoot in uninoculated,nodulated and mycorrhizal plants were observed at 40% level of fly ash, when compared with their respective controls. Increase in shoot fresh weight of uninoculated, nodulated and mycorrhizal plants observed at 60% level was not significant as compared to their respective controls (0% fly ash). In dual inoculated plants, shoot fresh weight was significantly reduced. Significant increase occurred in root fresh weight at 20% level in all the inoculated and uninoculated plants. Root fresh weight, however, showed decrease at 40% onwards in uninoculated, nodulated, dual inoculated plants in comparison to their respective controls. In mycorrhizal plants, however, significant increase in root fresh weight occurred upto 40%.Fly ash at 80 and 100% levels caused a significant decline in fresh weights of shoot and root in

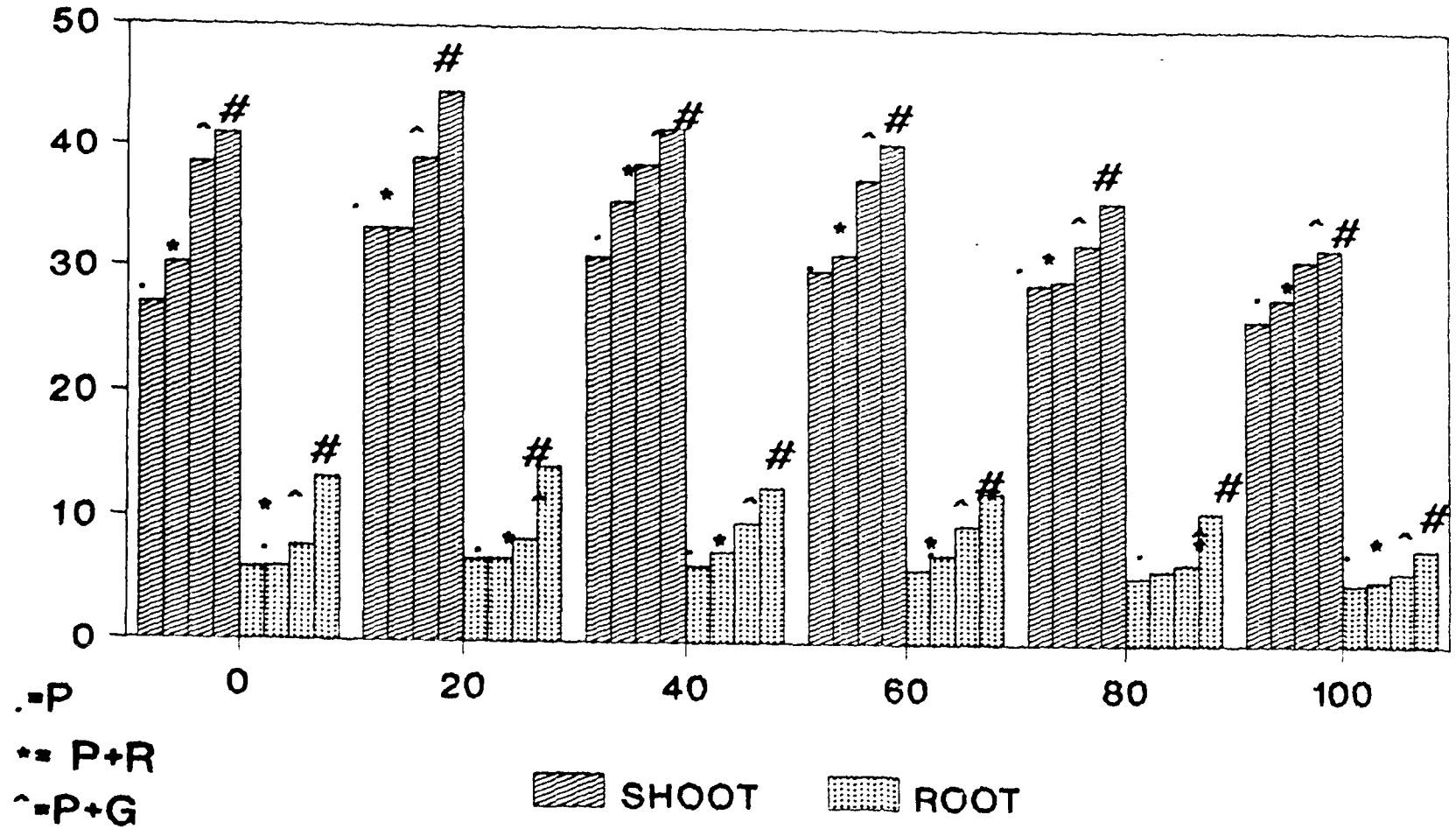
Table 11. Effect of soil amendment with fly ash on length of shoot and root of black gram plants inoculated with root symbionts.

Treatment		Fly ash(%)					
		Length(cm)					
		0	20	40	60	80	100
Plant (Black gram	S	27	33.2	31	30	29.0	26.2
without root	R	6	6.83	6.33	6.16	5.6	5.1
symbionts)							
Plant +	S	30.2	33.2	35.6	31.3	29.3	28
Rhizobium sp.	R	6.06	6.85	7.5	7.35	6.16	5.4
Plant + *Glomus*	S	38.6	39.1	38.7	37.5	32.3	31.1
caledonicum	R	7.8	8.4	9.78	9.70	6.7	6.13
Plant + *Rhizobium*	S	41	44.5	41.6	40.5	35.8	32.0
sp. + *G. caledonicum*	R	13.25	14.2	12.6	12.3	10.8	7.96

	C.D.(P=0.05)	
	S	R
Treatment	0.8987	0.5106
Fly ash	0.7338	0.4169
Interaction	1.797	1.0213

S =Shoot, **R** = Root

Each value is the mean of six replicates.



P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.24

uninoculated, nodulated and dual inoculated plants (Table 12; Fig. 25;Plate III).

Addition of fly ash in soil upto 60% level increased dry weights of shoot and root. Dry weights of shoot and root at 20, 40 and 60% levels of fly ash were significantly greater (P=0.05) in dual inoculated plants than single inoculated or uninoculated plants. Increases in shoot and root dry weights of mycorrhizal plants were greater than in nodulated plants. The increases were highest at 20% level in all treatments. Significant increase in dry weights of shoot and root also occurred at 40 and 60% levels of fly ash in uninoculated, mycorrhizal and nodulated plants. Dual inoculated plants showed an increase only in shoot dry weight at both levels, but their root dry weight declined. Higher levels of fly ash (80 and 100%) suppressed shoot dry weight in all the treatments and root dry weight in nodulated, mycorrhizal and dual inoculated plants (Table 13; Fig. 26)

Yield

The yield of black gram was affected by application of fly ash in soil. No pod formation occurred in uninoculated plants at 100% levels of fly ash. Dual inoculated plants produced greater number of pods than single and uninoculated plants at all levels of fly ash. Fly ash at 20 to 60% levels caused significant increase in pod numbers of uninoculated, nodulated and mycorrhizal, dual inoculated plants. At 80% it adversely affected the pod numbers in inoculated and uninoculated plants. At 100% level, significant reduction occurred in pod numbers of nodulated, mycorrhizal and dual inoculated plants (Table 14; Fig. 27).

The number of seeds per pod showed a significant enhancement upto 60% level of fly ash in uninoculated, mycorrhizal ,nodulated and dual inoculated plants in comparison to their respective controls. At 80% and 100% ,significant reduction (P=0.05) was observed both in inoculated and uninoculated plants (Table14;Fig.27).

Table 12. Effect of soil amendment with fly ash on fresh weight of shoot and root of black gram plants inoculated with root symbionts.

Treatment		Fly ash (%)					
		Fresh weight (g)					
		0	20	40	60	80	100
Plant (Black gram without root symbionts)	S	7.36	8.91	8.16	7.56	6.58	5.33
	R	1.38	1.50	1.33	1.26	1.15	0.99
Plant + *Rhizobium* sp.	S	8.10	10.0	9.78	8.79	6.77	5.60
	R	1.45	1.77	1.45	1.40	1.25	1.19
Plant + *Glomus caledonicum*	S	8.70	11.64	9.95	9.26	9.15	7.24
	R	2.40	2.61	2.48	2.24	2.22	1.62
Plant + *Rhizobium* sp.+ *G. caledonicum*	S	10.78	12.9	11.39	10.10	9.67	7.68
	R	2.95	4.10	3.53	3.38	2.36	2.31

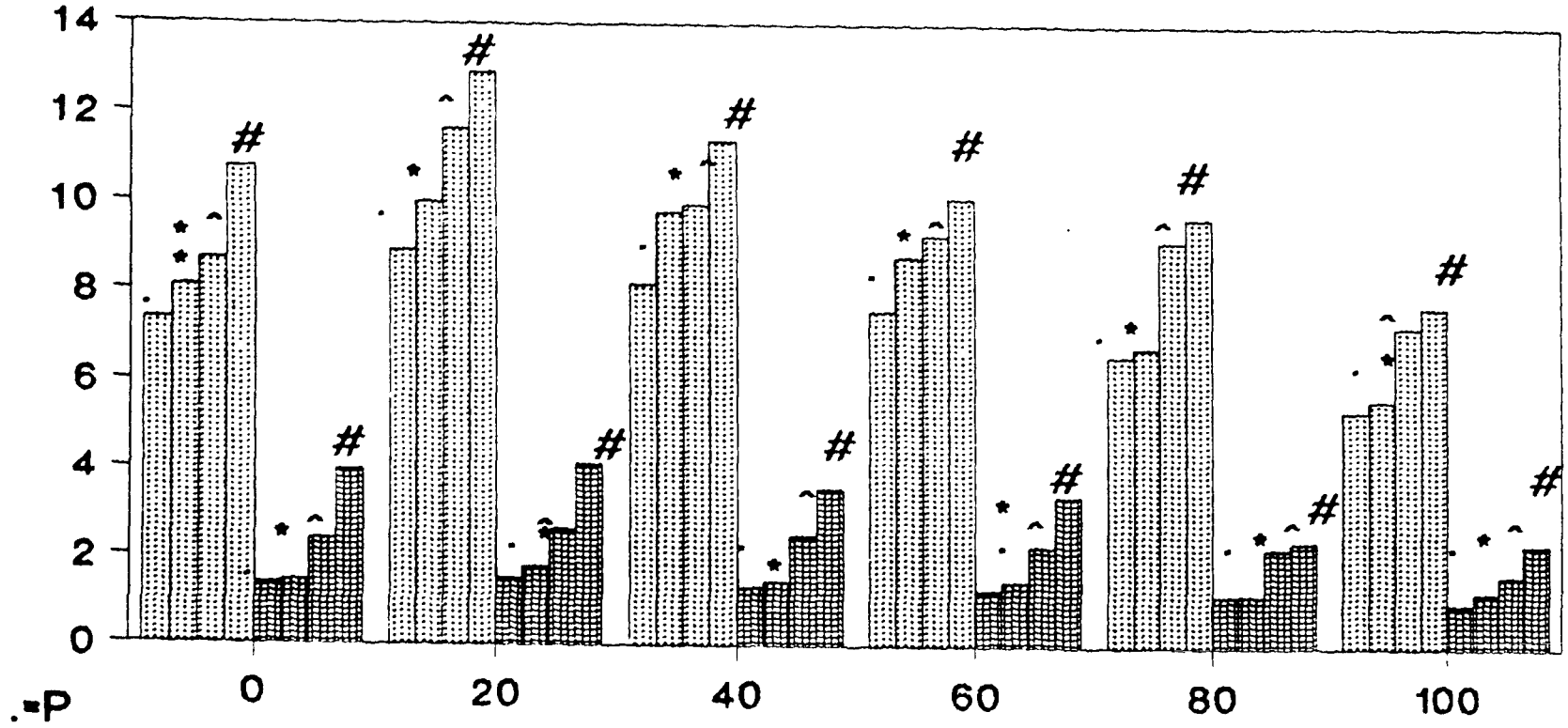
	C.D.(P=0.05)	
	S	R
Treatment	0.4469	0.1795
Fly ash	0.3649	0.1466
Interaction	0.89391	0.3591

S = Shoot, **R** = Root

Each vaule is the mean of six replicates.

FLYASH (V/V)

FRESH WEIGHT (g)



P=Plant. R = Rhizobium sp.G = Glomus caledonicum.

Fig.25

Plate No. III :- Plants growth in soil amended with fly ash (v/v).

1. Uninoculated plants.
2. *Rhizobium* inoculated plants.
3. *Glomus caledonicum* inoculated plants.
4. *Rhizobium* + *G. caledonicum* inoculated plants.


1


3

Table 13. Effect of soil amendment with fly ash on dry weight of shoot of black gram plants inoculated with root symbionts.

Treatment		Fly ash (%)					
		Dry weight (g)					
		0	20	40	60	80	100
Plant (Black gram without root symbionts)	S	2.86	4.0	3.66	2.75	1.93	0.89
	R	0.72	0.92	0.87	0.79	0.74	0.53
Plant + *Rhizobium* sp.	S	2.96	4.36	4.27	3.60	2.82	1.28
	R	0.79	0.95	0.94	0.90	0.76	0.59
Plant + *Glomus caledonicum*	S	3.26	5.18	4.61	3.86	2.96	1.67
	R	0.92	0.99	0.97	0.96	0.96	0.90
Plant + *Rhizobium* sp.+ *G. caledonicum*	S	5.19	6.68	6.27	5.89	3.86	1.80
	R	2.53	2.81	1.4	1.08	1.06	1.01

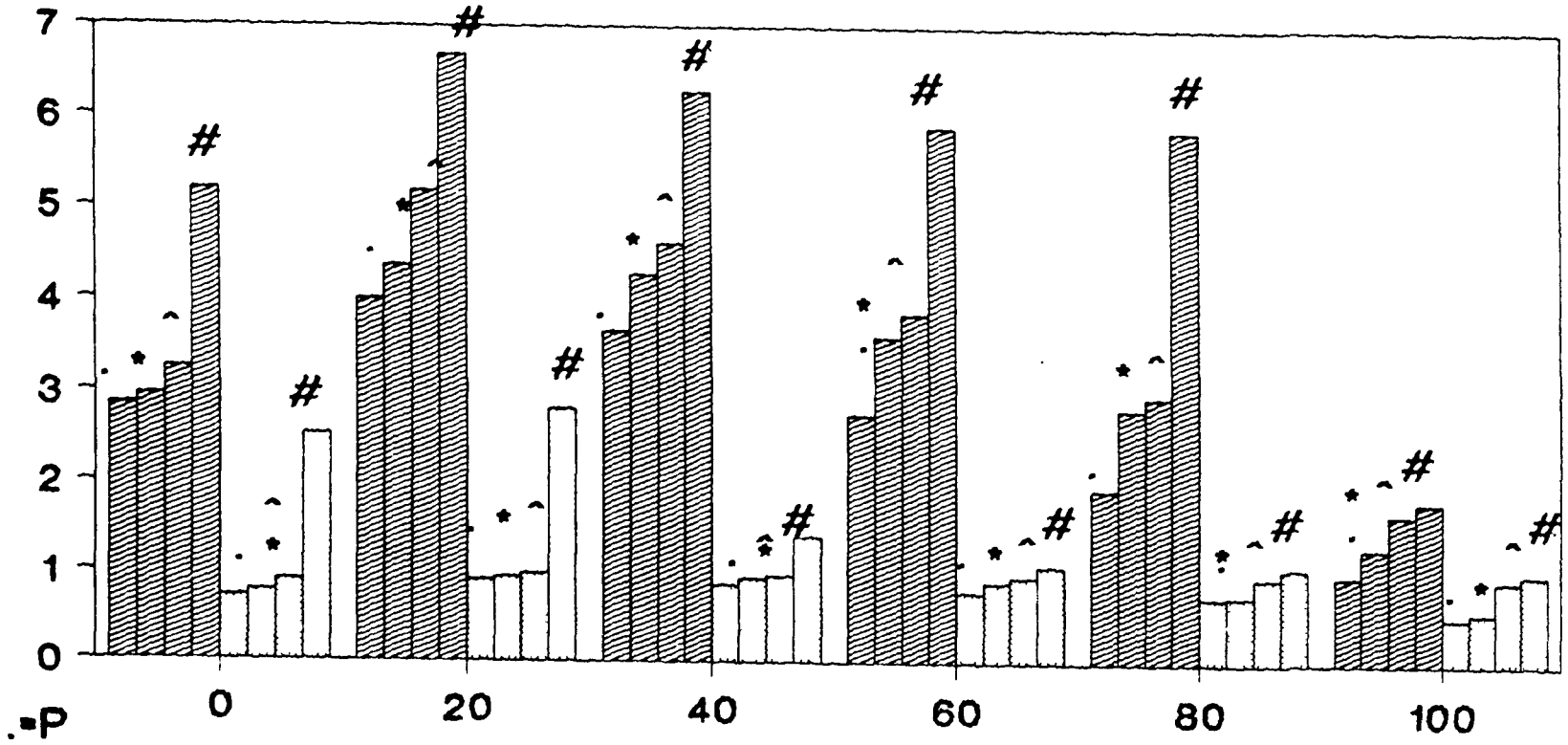
	C.D.(P=0.5)	
	S	R
Treatment	0.0573	0.0215
Fly ash	0.4685	0.0176
Interaction	0.1147	0.0431

S = Shoot, **R** = Root

Each value is the mean of six replicates.

FLYASH (V/V)

DRY WEIGHT (g)



P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.26

Table 14. Effect of soil amendment with fly ash on the yield of black gram plants inoculated with root symbionts.

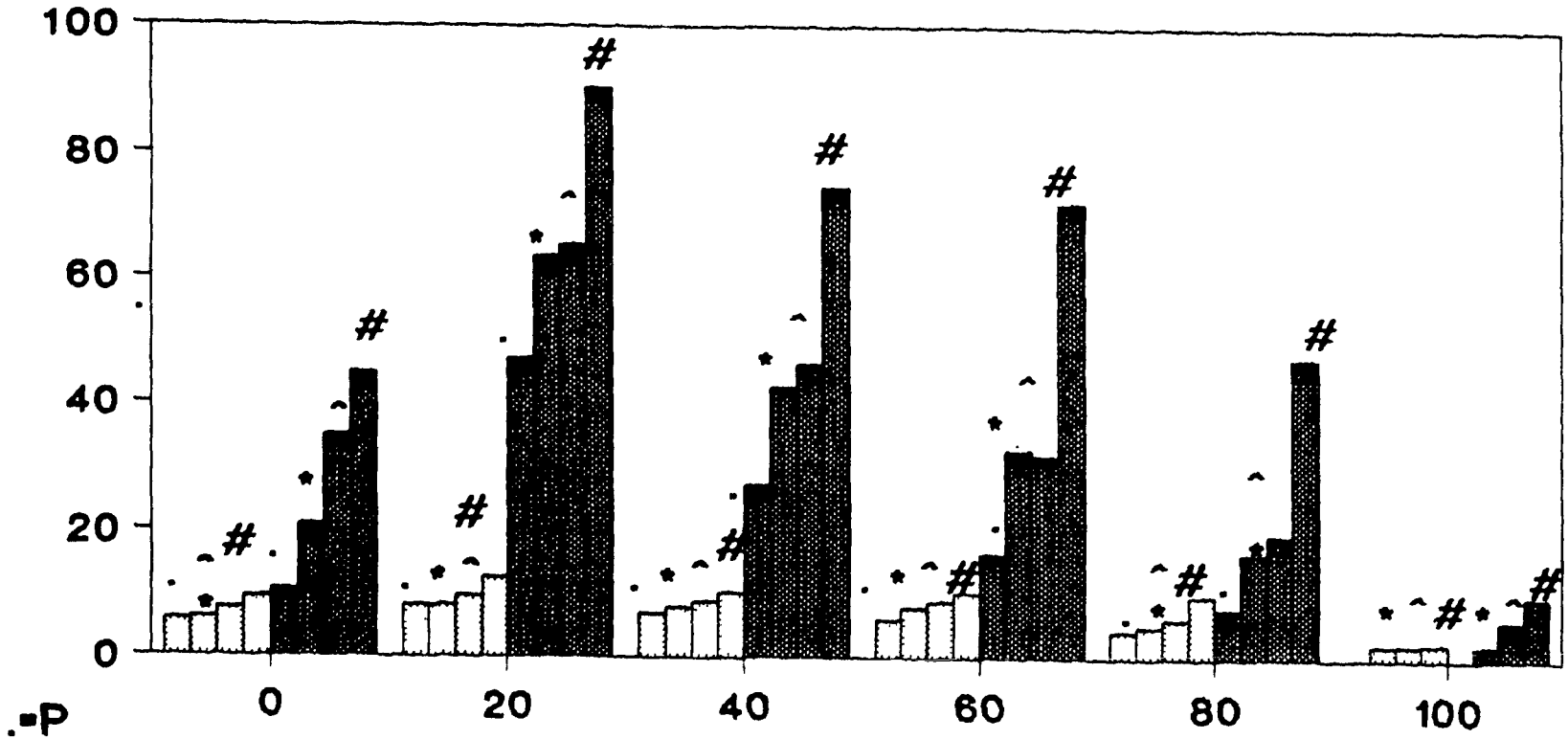
Treatment		Fly ash (%)					
		Yield					
		0	20	40	60	80	100
Plant (Black gram without root symbionts)	P.No.	6	8.33	7.16	6.16	4.33	
	S.No.	10.8	47.1	27.3	16.5	8	-
Plant + *Rhiz obium* sp.	P.No.	6.33	8.33	8.5	8.1	5.16	2.33
	S.No.	21	63.5	42.6	32.8	16.8	4.3
Plant + *Glomus caledonicum*	P.No.	7.83	9.8	9.6	9.1	6.33	2.33
	S.No.	35.1	65.3	46.3	37	19.6	6.16
Plant + *Rhiz obium* sp. + *G.caledonicum*	S.No.	9.6	12.8	10.8	10.8	10.1	2.6
	S.No.	44.8	90.3	74.6	72.1	47.3	10

	C.D.(P=0.05)	
	Yield	
	P.No.	S.No.
Treatment	0.64904	3.4079
Fly ash	0.52994	2.78260
Interaction	1.2980	6.81597

P.No.= Pod number, **S.No**.= Seed number / Pod.

Each vaule is the mean of six replicates.

FLYASH (V/V)
YIELD



P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.27

Leaf chlorophyll and seed protein

Inoculation of black gram plants with both root symbionts (singly or in combination) resulted in an increase in the chlorophyll (chlorophyll a, b and total chlorophyll) content of leaves and protein content of seeds. Dual inoculated plants contained greater chlorophyll and seed protein than single inoculated or uninoculated plants.

Chlorophyll content of leaves also showed an increase by the addition of fly ash in all the treatments. Significant increase (P=0.05), however, occurred in chlorophyll a, chlorophyll b and total chlorophyll at 20 and 40% fly ash. The dual inoculated plants showed greater chlorophyll content than single inoculated plants at all levels of fly ash .Significant decrease occurred in chlorophyll a in inoculated plants, at 60% level with exception of uninoculated plants in comparison to their respective controls (0% level). Chlorophyll b and total chlorophyll of uninoculated, nodulated, mycorrhizal and dual inoculated plants showed a significant reduction at 60% level when compared with their respective controls. At 80 and 100% levels, chlorophyll a , chlorophyll b and total chlorophyll of leaves were significantly (P=0.05) reduced in all the treatments (Table 15; Fig. 28 and 29).

Seed protein also showed a significant increase at 20 and 40% levels of fly ash in all the treatments in comparison to their respective control. Dual inoculated plants had greater seed protein at all levels of fly ash than single inoculated plants. At 60% level, reduction occurred in seed protein in uninoculated and mycorrhizal plant with exception of nodulated and dual inoculated plants in comparison to their respective controls. Further reduction occurred at 80 and 100% in all the treatments(Table 16;Fig. 30).

Root colonization, spore production and phosphorus content

Root colonization by the VAM fungus and spore number were greater in dual inoculated plants than single inoculated plants. Similarly phosphorus contents of shoot and root was also greater in dual inoculated plants than single inoculated plants.

Fly ash application suppressed root colonization both in single and dual inoculated plants at all levels of treatments. Spore number, however, increased significantly (P=0.05) at 20 and 40% levels in mycorrhizal and dual

Table 15. Effect of soil amendment with fly ash on chlorophyll content of leaf of black gram plants inoculated with root symbionts.

Treatment		Fly ash (%)					
		Chlorophyll (mg/g)					
		0	20	40	60	80	100
Plant (Black gram	a	0.010	0.018	0.015	0.011	0.010	0.009
without root	b	0.157	0.174	0.164	0.115	0.105	0.100
symbionts)	T	0.206	0.222	0.216	0.121	0.120	0.115
Plant +	a	0.013	0.019	0.018	0.012	0.011	0.010
Rhizobuim sp.	b	0.166	0.177	0.175	0.120	0.112	0.105
	T	0.211	0.254	0.249	0.126	0.121	0.119
Plant + *Glomus*	a	0.016	0.022	0.019	0.019	0.014	0.012
caledonicum	b	0.174	0.191	0.180	0.157	0.138	0.128
	T	0.218	0.260	0.254	0.129	0.126	0.123
Plant +	a	0.020	0.027	0.022	0.017	0.015	0.013
Rhizobuim sp. +	b	0.176	0.259	0.232	0.206	0.191	0.176
G. caledonicum	T	0.220	0.300	0.279	0.189	0.155	0.138

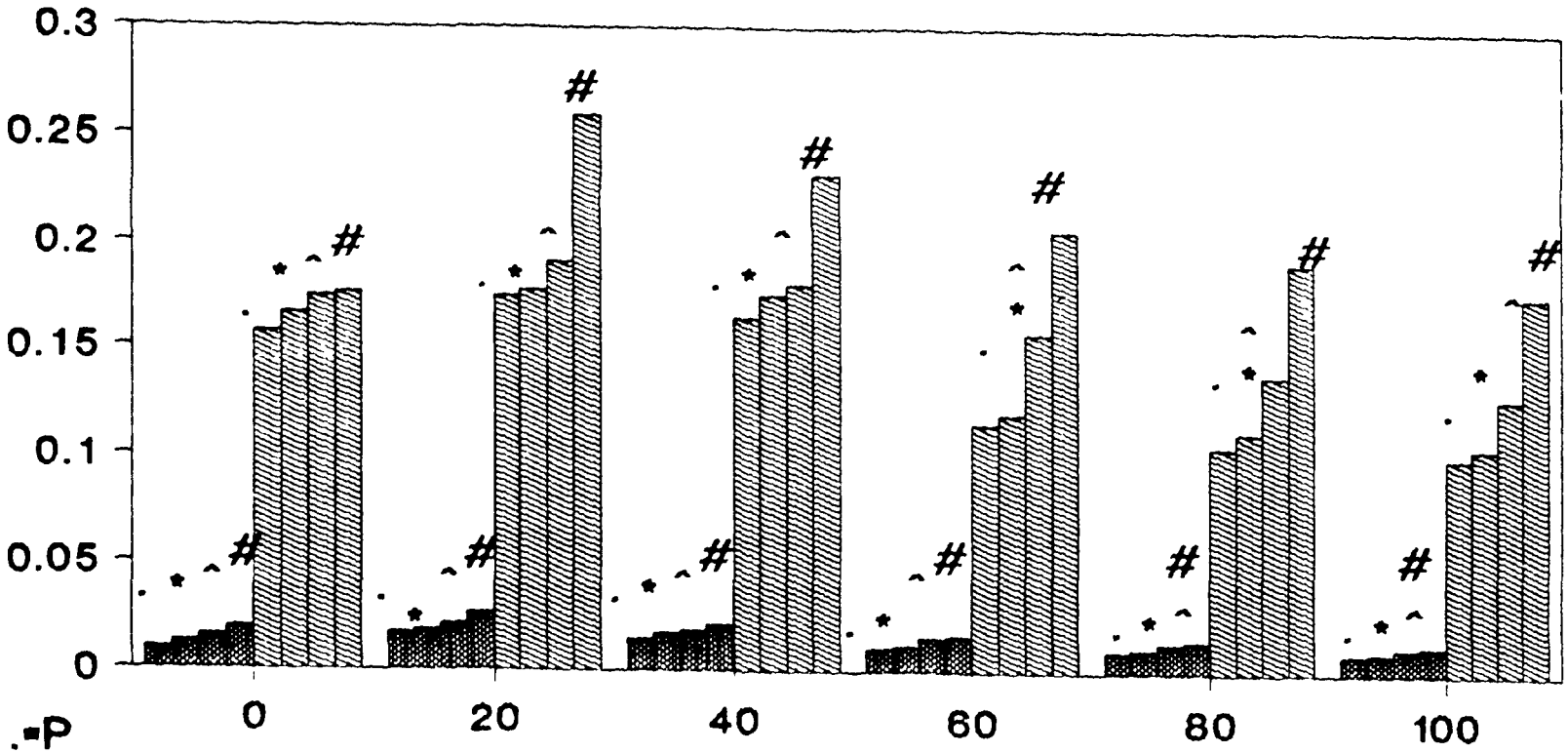
	C.D.(P=0.05)		
	a	b	T
Treatment	0.000977	(N.S)	0.00117
Fly ash	0.000797	0.04532	0.00095
Interaction	0.00195	0.91101	0.00232

a = Chlorophyll a, **b** = Chlorophyll b, **T** = Total chlorophyll

Each value is the mean of six replicates.

FLYASH (V/V)

CHLOROPHYLL (a,b) (mg/g)

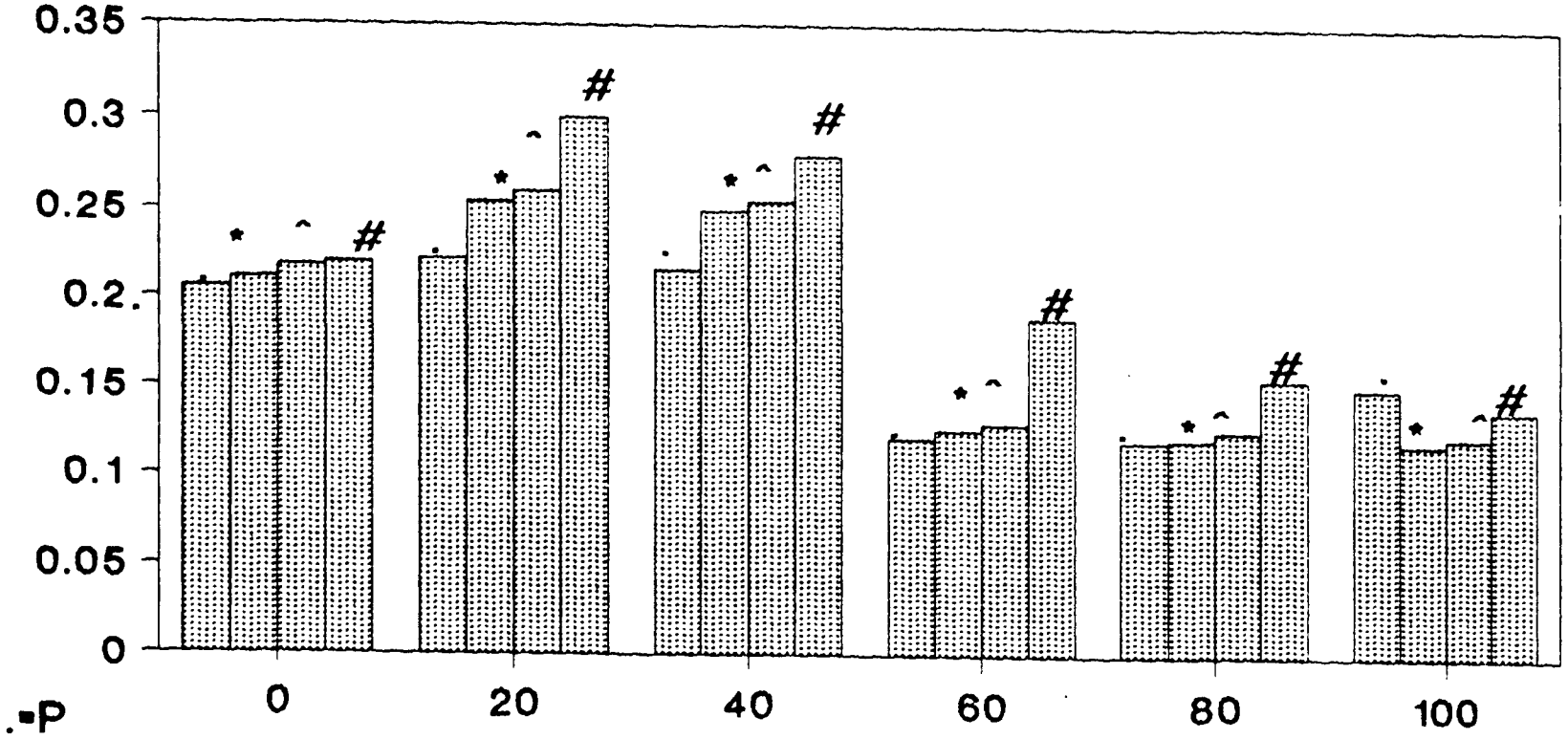


P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.28

FLYASH (V/V)

TOTAL CHLOROPHYLL (mg/g)



P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.29

Table 16. Effect of soil amendment with fly ash on seed protein of black gram plants inoculated with root symbionts.

Treatment	Fly ash (%)					
	Protein (%)					
	0	20	40	60	80	100
Plant (Black gram without root symbionts)	21.7	23	22.8	21.0	20.0	0.0
Plant + *Rhizobium* sp.	22.21	23.06	23.62	22.53	21.8	18.64
Plant + *Glomus caledonicum*	22.89	23.92	23.66	22.60	21.9	19.54
Plant + *Rhizobium* sp. + *G. caledonicum*	24.87	26.47	26.26	25.31	23.9	21.89

	C.D.(P=0.05)
Treatment	0.61331
Fly ash	0.5007
Interaction	1.2266

Each value is the mean of six replicates.

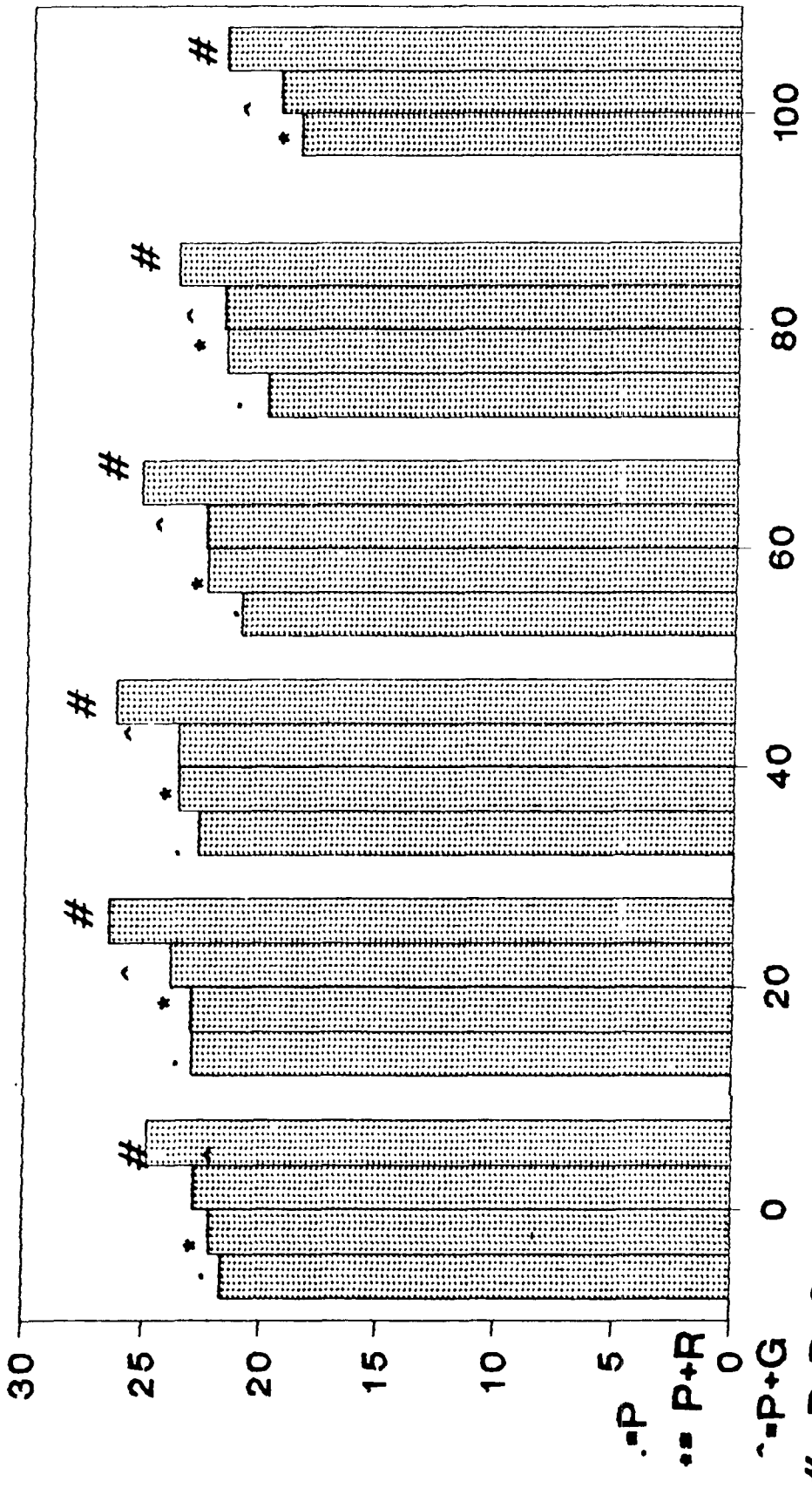

FLYASH (V/V)
PROTEIN (%)
30
25
20
15
10
5
0
0
20
40
60
80
100
.=P
*= P+R
^=P+G
#= P+R+G P=Plant. R = Rhizobium sp.G = Glomus caledonicum.

Fig.30

inoculated plants in comparison to their respective controls. A decline in spore number was observed from 60% onward upto 100% level of fly ash (Table 17;Fig. 31).

Significant increase in phosphorus contents of shoot and root occurred at 20 and 40% in all the treatments when compared with their respective controls. At 60% level of fly ash, phosphorus contents of root and shoot was reduced significantly in uninoculated, nodulated and mycorrhizal plants, except in the shoot of dual inoculated plants in which an increase occurred . At 80 and 100% levels, significant reduction occurred in all the treatments (Table 18;Fig. 32).

Root nodulation and nitrogen content

Nodule number and dry weight of nodules were greater in dual inoculated plants than single inoculated plants with *Rhizobium* sp. Nitrogen contents of shoot and root of dual inoculated plants were also greater than single inoculated plants.

Application of fly ash caused a significant increase in nodule number, dry weight of nodules (P=0.05) upto 40% level in both treatments (single and dual inoculations). Fly ash significantly caused reduction (P=0.05) in nodule number and in their dry weight from 60 to 100% levels in dual and single inoculated plants. At 100% level, the reduction in nodule number and dry weight of nodules were greater than 60% or 80% levels (Table 19; Fig. 33).

Nitrogen contents of shoot and root were significantly enhanced by the fly ash at 20 and 40% levels. At 60% ,shoot nitrogen of uninoculated plants was equal to uninoculated control (without fly ash) plants. Significant reduction occurred in nitrogen contents of shoot and root in nodulated and mycorrhizal plants at 60% level, except root and shoot nitrogen of dual inoculated plants which showed an increase. At 80% and 100% levels of fly ash, in all the treatments (uninoculated , single or dual inoculated) significant reduction occurred in comparison to their respective controls (Table 20; Fig. 34).

Table 17. Effect of soil amendment with fly ash on root colonization and spore number of VAM fungus, *Glomus caledonicum* on black gram plants inoculated with root symbionts.

Treatment		Fly ash (%)					
		Root colonization / Spore number					
		0	20	40	60	80	100
Plant (Black gram without root symbionts)		-	-	-	-	-	-
Plant + *Rhizobium* sp.		-	-	-	-	-	-
Plant + *Glomus caledonicum*	Co	58.1	57.8	56.6	54.0	51.8	38.5
	C.No.	370	380	375	368	300	210
Plant + *Rhizobium* sp. + *G.caledonicum*	Co	73.8	71.8	71.6	65.1	64.0	42.6
	C.No.	401	41.6	411	391	350	300

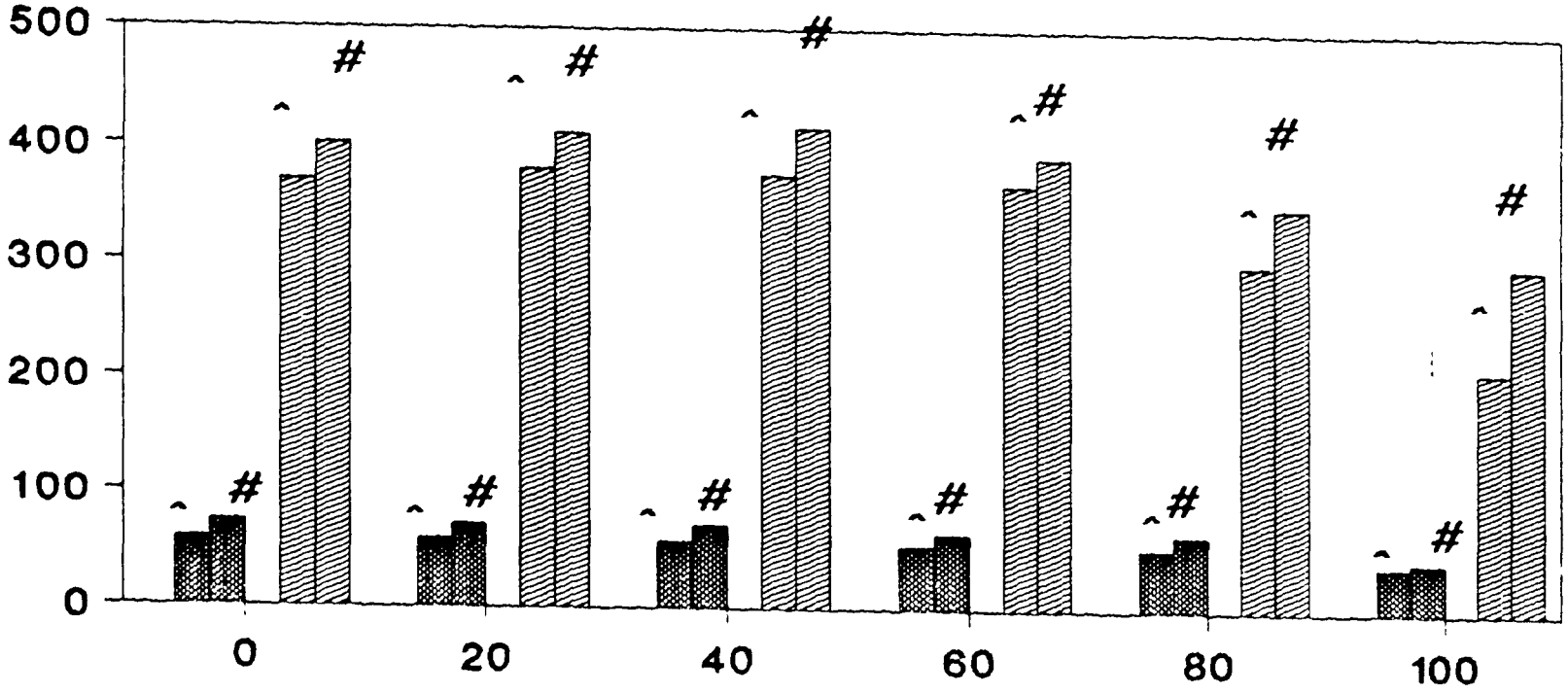
	C.D.(P=0.05)	
	Root colonization	Spore number
	CO	C.No.
Treatment	0.5797	3.6712
Fly ash	0.47254	2.9975
Interaction	1.15748	7.342

Co = % Root colonization , **C.No**. = Spore number.

Each value is the mean of six replicates.

FLYASH (V/V)

ROOT COLONIZATION AND SPORE NUMBER OF VAM FUNGUS



^=P+G

#= P+R+G

P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.31

Table 18. Effects of soil amendment with fly ash on phosphorus content of shoot and root of black gram plants inoculated with root symbionts.

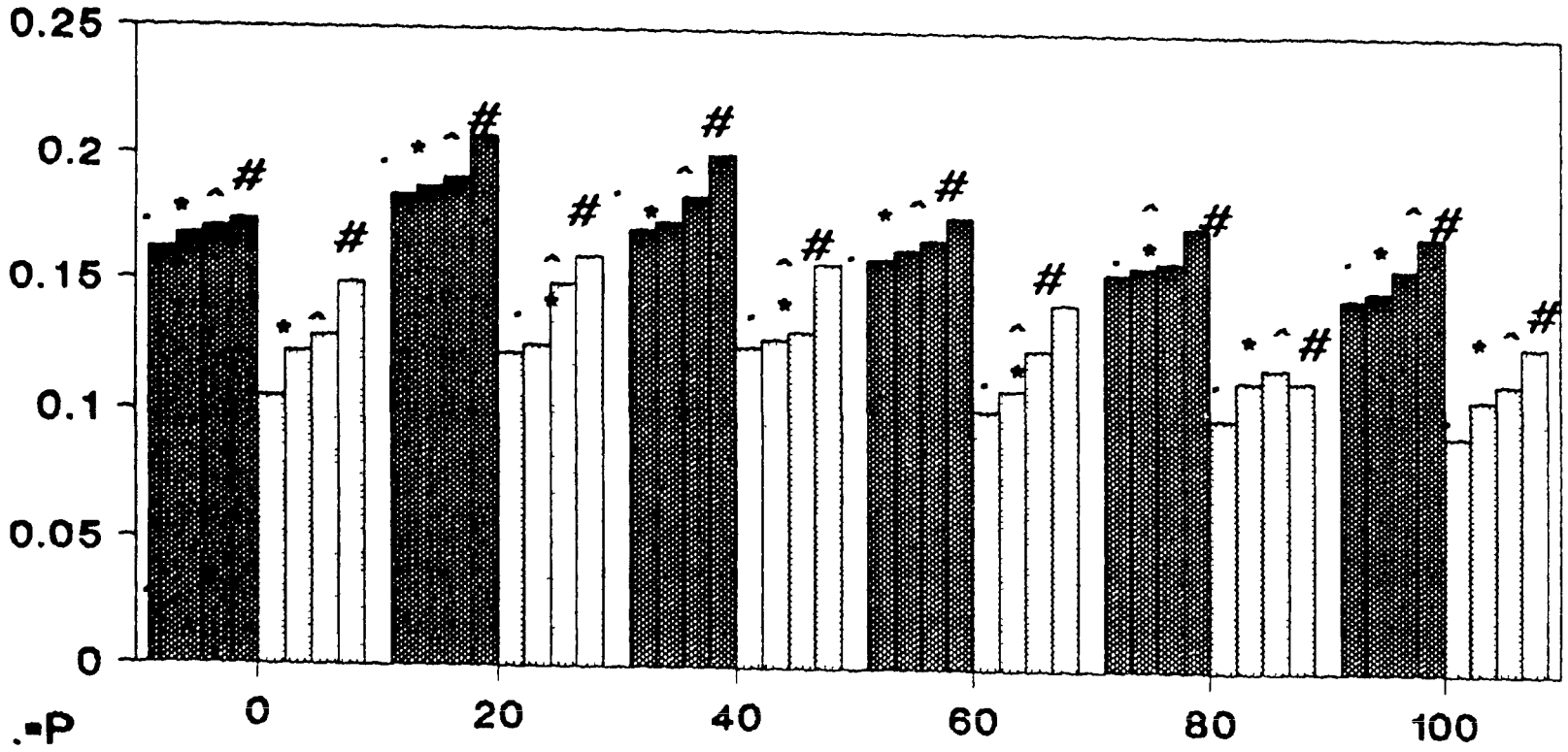
Treatment		Fly ash (%)					
		Phosphorus (%)					
		0	20	40	60	80	100
Plant (Black gram without root symboints)	S	0.162	0.184	0.171	0.160	0.155	0.146
	R	0.105	0.122	0.125	0.102	0.100	0.094
Plant + *Rhizobium* sp.	S	0.168	0.187	0.174	0.164	0.158	0.149
	R	0.122	0.125	0.128	0.110	0.114	0.108
Plant + *Glomus caledonicum*	S	0.171	0.191	0.184	0.168	0.160	0.158
	R	0.128	0.149	0.131	0.125	0.119	0.114
Plant + *Rhizobium* sp. + *G.caledonicum*	S	0.174	0.208	0.201	0.177	0.174	0.171
	R	0.149	0.160	0.158	0.143	0.134	0.128

	C.D.(P=0.05)	
	S	R
Treatment	0.00798	0.00112
Fly ash	0.00651	0.00092
Interaction	0.01596	0.00225

S = Shoot, **R** = Root

Each value is the mean of six replicates.

FLYASH (V/V)
PHOSPHORUS (%)



P=Plant. R = Rhizobium sp.G = Glomus caledonicum.

Fig.32

Table 19. Effect of soil amendment with fly ash on nodule number , nodule dry weight of black gram plants inoculated with root symbionts.

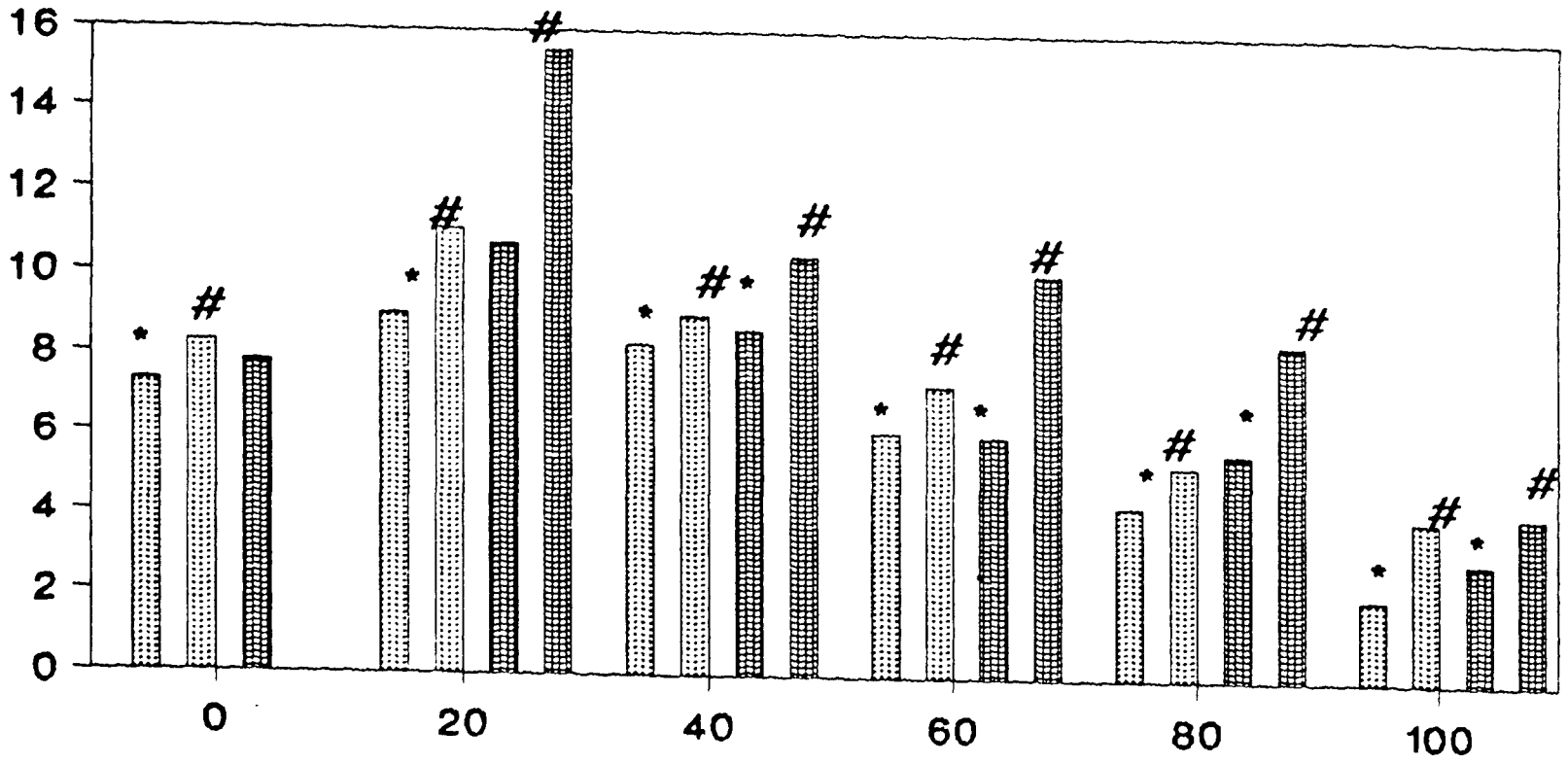
Treatment		Fly ash (%)					
		Nodule number / Nodule dry weight (mg)					
		0	20	40	60	80	100
Plant (Black gram without root symbionts)		-	-	-	-	-	-
Plant + *Rhizobium* sp	N.No.	7.3	9	8.3	6.1	4.3	2
	D.W	7.8	10.73	8.66	6.01	5.63	2.96
Plant + *Glomus caledonicum*		-	-	-	-	-	-
Plant + *Rhizobium* sp + *G.caledonicum*	N.No.	8.3	11.1	9	7.3	5.3	4
	D.W.	12,o5	15.5	10.48	10.08	8.41	4.16

	C.D.(P=0.05)	
	Nodule number	Dry weight
	N.No.	D.W.
Treatment	0.41937	0.4329
Fly ash	0.3424	0.3535
Interaction	0.8387	0.8659

N.No.= Nodule number, **D.W**.= Dry weight of nodules

Each value is the mean of six replicates.

FLYASH (V/V)
ROOT NODULATION



* = P+R NODULE NUMBER DRY WEIGHT OF NODULE

= P+R+G

P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.33

Table 20. Effect of soil amendment with fly ash on nitrogen content of shoot and root of black gram plants inoculated with root symbionts.

Treatment		Fly ash (%)					
		Nitrogen (%)					
		0	20	40	60	80	100
Plant (Black gram	S	0.160	0.184	0.177	0.160	0.152	0.146
without root	R	0.114	0.119	0.116	0.111	0.102	0.095
symbionts)							
Plant +	S	0.174	0.197	0.187	0.171	0.155	0.149
Rhizobium sp.	R	0.119	0.125	0.122	0.114	0.108	0.102
Plant + *Glomus*	S	0.191	0.208	0.201	0.181	0.177	0.155
caledonicum	R	0.122	0.128	0.125	0.119	0.114	0.105
Plant +	S	0.201	0.256	0.244	0.204	0.187	0.177
Rhizobium sp. +	R	0.125	0.194	0.191	0.181	0.155	0.116
G.caledonicum							

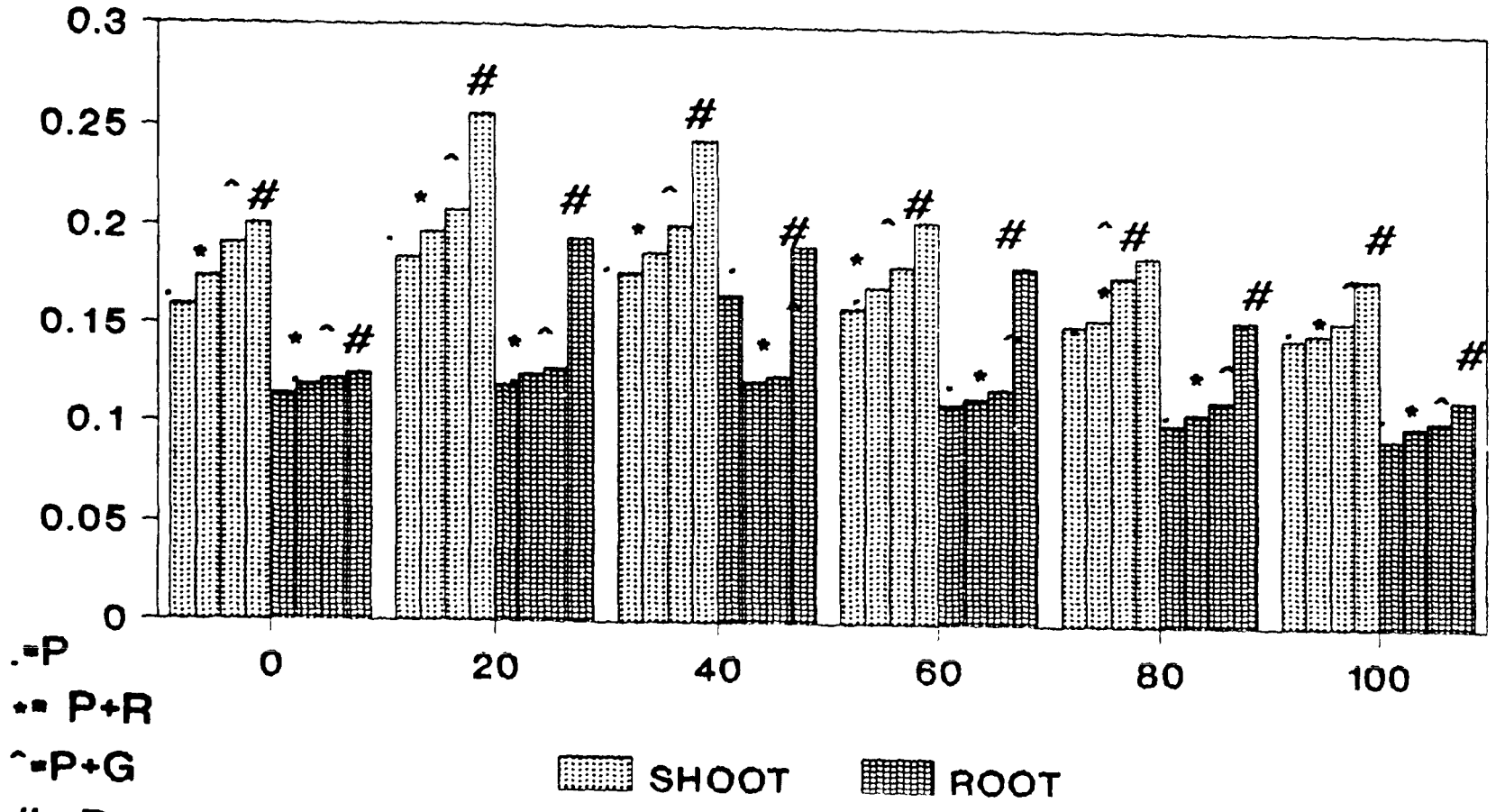
	C.D.(P=0.05)	
	S	R
Treatment	0.00475	(N.S)
Fly ash	0.00388	0.03595
Interaction	0.00951	0.08807

S = Shoot, **R** = Root

Each value is the mean of six replicates.

FLYASH (V/V)

NITROGEN (%)



P=Plant. R = Rhizobium sp. G = Glomus caledonicum.

Fig.34

DISCUSSION

Air pollution is becoming increasingly serious threat for all living organisms.Agricultural crops grown in crop fields in the vicinity of point sources particularly industries using fossil fuels seem to be in real danger. Various life processes of plants from seed germination to flowering and fruiting are likely to be affected by different air pollutants emanating from such industries. Root symbionts, root nodule bacteria and VAM fungi,are beneficial organisms for crop plants. With leguminous crop, both the groups of microorganisms develop symbiotic relationship and depend on the crop for their sustenance while providing benefits to the crops. These root symbionts and their symbiotic relationship and the mutual benefits derived from such associations may be influenced on air pollution-stressed plants. The present study aimed to determine the influence of two important gaseous air pollutants namely SO_2 and O_3 on these two root symbionts, selecting a leguminous crop black gram, commonly grown in India. The influence of fly ash of thermal power plant origin which accumulates in the soil gradually in the fields around the point source was also determined by adding it artificially to the soil for the experiment. The experiments were conducted under artificial treatment conditions in glasshouse. The treatment patterns were different for each air pollutant. The experiments were designed to approximate the levels of gaseous air pollutants in ambient air and the levels of fly ash present in the soil around the coal-fired thermal power plant like the one located at Kasimpur about 15 km away from the Aligarh Muslim University Campus. This thermal power plant has been used in some studies (Singh,1989,1993; Pasha,1991) to determine the impact of ambient air pollution on crop productivity and plant diseases caused by root-knot nematodes and powdery mildews.

Sulphur dioxide (SO_2)

Plants growth and yield of black gram inoculated either with root nodule bacterium, *Rhizobium* sp. or VAM fungus, *Glomus caledonicum* or both showed great improvement. Root nodule bacteria through nitrogen fixation , provides nutritional benefits to leguminous plants.*Rhizobium* sp.formed root nodules on black gram roots for

symbiotic nitrogen fixation which may have improved its plants growth and yield. Uptake of phosphorus might have enhanced by *G.caledonicum* as mycorrhizal fungi increase the total surface area in contact with soil and mobilize phosphorus. Uptake of other nutrients like Zn,Ca,Mg,S,Fe and other ions is also increased by VAM fungi (Sanders *et al.*, 1977; Rhoads and Gerdemann,1978; Copper and Tinker,1978). Improvement in growth and yield of mycorrhizal black gram plants, therefore, was obviously due to greater uptake of phosphorus and other elements. Growth and yield of black gram plants inoculated with both root symbionts (dual inoculation) were much greater than the plants inoculated either with root nodule bacterium or VAM fungus. Adequate supply of nitrogen through nitrogen fixation by *Rhizobium* sp. and supply of phosphorus and other elements through mycorrhizal roots developed by *G.caledonicum* created better nutrient availability conditions in comparison to plants inoculated singly with either symbiont. Therefore, presence of both root symbionts simultaneously resulted in highest growth and yield by the plants.It seems that the leguminous plants can derive maximum benefits concurrently through symbiosis by these two diverse groups of microorganisms. These attributes are, however ,restricted to leguminous crops since other crops in general do not develop symbiotic association with root nodule bacteria. Similar results in relation to effect of dual and single inoculation by root nodule bacteria and VAM fungi on leguminous crops have been reported by Adholeya *et al.* (1988, a,b) and Jahan (1993).

Sulphur dioxide suppressed growth of black gram but yield was not significantly reduced. Sulphur dioxide in general adversely affects vital physiological and biochemical processes of plants and causes injury to tissues, cell and cell organelles, particularly in the foilage (Carlson, 1983). Black gram plants exposed to SO_2 concentrations also showed injuries and could have responded in a way by which physiological and biochemical processes of the exposed plants could have been impaired resulting in suppressed growth. In recent years plants growth of several crop plants including legumes have been reported to be adversley affected by SO_2 exposures. Soybean (Sprugel *et al.*, 1980), *Phaselous* sp. (Saxe ,1983),lentil and chickpea (Singh,1989),*Medicago* sp.(Murrary and Wilson, 1991), cucumber (Pasha, 1991), tomato (Khan and Khan, 1993), okra and egg plant

(Khan and Khan, 1994 a,1994b) are some of plants reported to exhibit suppressed plants under SO_2 pollution.SO_2 exposure, however, did not cause significant reduction in the yield of black gram assessed on the basis of number of pods/plant and number of seeds/pods. Some reports indicate that the yield is not always adversely effected by SO_2 exposure particularly at lower concentrations, although the plants growth is suppressed (Lotstein, *et al.*, 1983;Murray and Wilson, 1990; Adaros *et al.*, 1991). Exposure of tomato plant to 0.1 ppm of SO_2 for 72h/ week for 5 to 10 weeks had no effect on fruit yield and othere soluble solid *content in the ripe fruits (Lotstein et al., 1983). Murray and Wilson* (1990) observed that SO_2 reduced the number of mature pods and kernels in peanut but the weight of kernels produced was unchanged. They also showed that in navy beans,SO_2 (290 ug/m3) increased the number of pods and beans per plant, however, average bean weight was reduced resulting in no net changed in the yield.Yield was increased by exposure to 141 ug/m3 of SO_2. Sulphur concentration increased in the leaves at 280 ug/m3 but remained unaffected by 140 ug/m3. They suggested that increase in sulphur in leaves at higher dosage and no increase at lower dosage indicate that it may have overcome marginal nutritional deficiency. Its accumulation might have stressed the metabolic processes reducing the pod and seed production. Therefore, it becomes evident that concentration of air pollutant is an imporatant determinant of the response of the plants in relation to thier yields. In the present study, the concentrations of SO_2 used were low (0.05 and 0. 1 ppm) and therefore, it did not affect the yield of black gram significantly, although plant growth was suppressed. Possibly the black gram may have a degree of tolerance to SO_2 in exhibiting its reduced productivity. In some studies (Singh, 1989; Singh,1992) suppression of both plant growth and yield of leguminous plants occurred. The concentrations of SO_2 used in those studies for exposure of plants were 2 to 4 times greater than those used in the present study.Plant growth of nodulated, mycorrhizal and dual inoculated plants were less affected by SO_2 exposures compared to uninoculated exposed plants.Apparently better nutritional status of such plants achieved through symbiotic associations provided partial protection to the plants from the adverse effcts of SO_2.This implies that leguminous plants with symbiotic association could grow better in SO_2

polluted areas than non-symbiotic plants. Out of three symbiotic situations involved in the experiment,dual inoculated plants were better and could escape to a great extent from the adverse impact of SO_2.Therefore,root nodule bacteria and mycorrhizal fungi can be introduced through artificial inoculation for growing leguminous crops under air pollution-stressed condition to alleviate adverse impacts.The repeatability of the results even with O_3 treated plants during the study,further support this contention that root symbionts are beneficial to leguminous crops even under air pollution-stressed condition and ensure better crop growth than non -symbiotic leguminous plants or non- leguminous crops .

Leaf chlorophyll of the exposed plants of black gram was significantly reduced.Destruction of phaeophytinization of chlorophyll molecules are attributed as causes for reduction of leaf chlorophyll in plants growing under SO_2 pollution (Rao and LeBlanc ,1966; Laurenorth and Dodd,1981). Entry of SO_2 into the leaves through stomata and its conversion into sulphite ions by reacting with water in leaves tissues are well established (Thomas *et al.*,1944). These sulphite ions when accumulate in excess cause destruction or phaeophytinization of chlorophyll molecules and reduce chlorophyll content of leaves and influence vital metabolic processes and enzymatic activities of plants adversely (Varshney and Garg,1979; Pierre and Qurieoz,1982).Destruction of chlorophyll molecules and / or their phaeophytinization and inhibition or interference in vital metabolic and enzymatic activities individually or collectively may be responsible for reduction in chlorophyll content.The chlorophyll content of leaves of nodulated,mycorrhizal and dual inoculated plants exposed to SO_2 was greater than non- symbiotic exposed plants. Like plant growth,the better nutrient status of such plants partially alleviated the adverse effect of SO_2 on chlorophyll.Seed protein in black gram was also reduced by SO_2 exposure.Direct interference of the air pollutant in the metabolic activities of plants related to protein synthesis or indirect effect through poor plant growth may be the causes for reduction in seed protein (Craker,1972; Khan and Malhotra,1983;Jahan,1993).

Sulphur dioxide inhibited root colonization of black gram plants and spore production by VAM fungus in all the treatments.The plants

stress,therefore,may have contributed towards inhibition in nodulation and subsequent reduction in nitrogen content of shoot and root .

Possibly ,the improved nutrition of mycorrhizal and nodulated plants and enhanced photosynthesis due to increase in chlorophyll content promoted plant growth and other determined parameters of black gram and this impact of the root symbionts minimized the negative effects of SO_2.SO_2 , however,suppressed root nodulation and root colonization by the VAM fungus and benefits derived from the root symbionts were therefore,reduced.But black gram plants inoculated with the root symbionts.SO_2 caused suppressive effects on the plants directly as well as indirectly through inhibition of the root symbionts and there symbiotic activities.Dual inoculated plants in general performed best, compared to the plants inoculated with either of the root symbionts.Therefore,it can be derived that root symbionts are capable of alleviating to a great extent,adverse effects of SO_2 on plants. This is an additional attribute of the root symbionts which can be agriculturally exploited under SO_2 pollution conditions.

OZONE (O_3)

Like sulphur dioxide, O_3 also suppressed plant growth to black gram in both the treatment patterns designed for the study to approximate ambient conditions. These effects were concentration dependent and higher concentration of O_3 (0.05-0.1-0.05 ppm) was more suppressive than the other treatment (0.02-0.05-0.02 ppm). Significant reduction occurred in all the considered plant growth parameters in both the treatments. O_3 treatments were also effective in suppressing the yield of the black gram plants. Adverse effect of O_3 on plant growth and / or yield has been observed in a number of studies. Inhibition in growth rate and pod production of snapbean (Blum and Heck, 1980), reduced plant growth of clover (Letchworth and Blum, 1977), suppression of clover regrowth and root and shoot growth (Blum *et al.*,1983 a,1983 b), reduction in plant growth of chickpea and lentil (Singh, 1989) and adverse effect on plant growth and yield of soybean (Singh, 1993) and mungbean and chickpea (Jahan, 1993) have been observed. In the present study, reduction in the yield may have resulted from poor plant health due to exposures. This declined conditions of the

black gram plants may have directly and adversely affected the flowering and fruiting, leading to yield loss. O_3 is known to be responsible for shedding pollen grains, suppressing their germination and pollen tube growth and inactivation of ovules (Kress *et al.*,1986).Fertilization failures are also caused by air pollutants (Linzon, 1978). These adverse effect may have conjointly reduced the number of pods and seeds in the pods produced on exposed plants of black gram.

Like earlier experiment, inoculation of the plants either singly or in combination with *Rhizobium* sp. and *G.caledonicum* enhanced plant growth and yield of black gram. Dual inoculation was most effective, which confirmed that presence of the two symbionts at the roots with symbiotic relationship can jointly provide greater benefit to the plants than the either symbiont. This, however, is practically possible with leguminous crops alone because of specificity in symbiosis shown by *Rhizobum* sp. Root nodule bacteria are well known for symbiotic nitrogen fixation which are beneficial for growth and yield of leguminuous plants (Taha, 1993). VAM fungi increase phosphorus nutrition and improve plant growth (Hayman and Mosse, 1972; Hayman, 1978). Even under O_3 stress by which root nodulation and root colonization by the symbionts were affected adversely, the root symbionts had favourable effects on the crop. In nodulated, mycorrhizal or dual inoculated plants, the suppressive effects of O_3 were comparatively much less than the exposed plants without root symbionts. Therefore, it emerges that yield losses of leguminous crops caused by O_3 is in natural conditions can be reduced by the presence of the root symbionts. This can be achieved and adverse effect can be minimized by such symbiotic associations by treating the plants artificially by the root symbionts. These two kinds of root symbionts also interact synergistically in providing benefit to the crops (Manjunath, 1984; Murakami *et al.*, 1991).

Leaf chlorophyll was also significantly reduced by O_3 treatments. The symbionts through better health of the plants, achieved by symbiotic associations, reduced the adverse effect of O_3 on chlorophyll content. Similar results were reported by Jahan (1993) and Singh (1989). Discolouration caused by destruction of chlorophyll results from accumulation of O_3 in palisade layer of the leaves. The affected

palisade cells ultimately collapse (Macdowall, 1965; Sakaki *et al.*, 1985). O_3 induced inhibition in electron transport system of chloroplast has been reported by Rhoads and Brennan (1978). They observed that inhibitory effect was greater in sensitive cultivar than resistant cultivar of tobacco. O_3 also affects structure and physiology of cell membrane, resulting in leakage of electrolytes (Rhoads and Brennan, 1978, Heck *et al.*, 1986). The reduced chlorophyll content through such destruction is likely to affect the photosynthesis which eventually lead to poor growth and yield of the plants. This might have been the case with black gram plants exposed to O_3.

Root nodulation in relation to nodule number and dry weight of nodules, and nitrogen content of black gram plants exposed to both the treatments of O_3 were reduced. Direct effect of O_3 on nodulation can be possibly ruled out to a great extent because O_3 molecules break down immediately after penetration in the soil. The adverse impact of O_3 on root nodulation was, therefore, indirect and host-mediated. Nutritional and physical factors both, may have contributed towards reduction in root nodulation. Reduced translocation of photosynthates to roots (Tingey, 1974) and poor root growth may have not sustained *Rhizobium* sp. adequately. Suppression in root nodulation and declined nitrogen fixation have been reported for a number of leguminous crop like soybean (Tingey and Blum, 1973; Singh, 1993), lentil and chickpea (Singh, 1989) and mungbean and chickpea (Jahan, 1993). Tingey and Blum (1973) correlated inhibition in root nodulation by *Rhizobium* on soybean to reduced carbohydrates in roots caused by O_3 exposures. Root nodulation is correlated with nitrogen fixation and consequently nitrogen content of the plants. Therefore, reduction in nodulation directly affects the nitrogen content. Decline in the phosphorus content of the plants may also be an important limiting factor for nitrogen fixation. Phosphorus plays a vital role in energy transfer and large quantity of energy is requried during nitrogen fixation. Application of phosphorus to soil usually increases and P and N content of the plant tissues (Andrews, 1976). In the present study, presence of *Rhizobium* sp. and *G. caledonicum* on the roots of black gram as symbionts improved the N and P contents. The improvement of P uptake by the plants as a result of VAM infection enhances nodulation and nitrogen fixation (Manjunath *et al.*, 1984). Enhanced absorptive

surface area of mycorrhizal roots extracts phosphorus from a greater volume of soil (Hayman and Mosse, 1972). The VAM fungus alone or with root nodule bacterium in the present study, may have compensated for the reduction in the root surface area caused by O_3. The VAM fungus may have also enhanced the efficiency of P uptake. Root colonization by VAM fungus and the spore count were also suppressed on black gram exposed to O_3. Phosphorus content was also affected adversely in both the treatments. Fiecht (1981) found decline in spore production of VAM fungus due to treatment with O_3 but root colonization was not affected. The effect of O_3 on VAM fungus was claimed as host- mediated through host metabolism, rather than direct (Blum and Tingey, 1977). Reduction in carbohydrate contents of the roots by O_3 may have affected the spore count of *G. caledonicum*. The decreased root carbohydrate may have resulted decreased carbon allocation to the roots (McCool, 1982) which as a whole reflects an integrated response that links photosynthesis to growth. The host-fungus compatibility might have been affected adversely through altered root carbohydrate allocation under O_3 stress.

The results of the present study indicate that all the three components of the system were adversely affected by O_3. Adverse effect of O_3 on plants were partially reduced by the symbiotic associations of root nodule bacterium and VAM fungus. Such results are expected for other leguminous crops in the presence of the root symbionts under O_3 pollution. The root symbionts, therefore, are not only beneficial under pollutant free environment but are also important for alleviation of adverse impacts of O_3 and SO_2 pollution as observed in the experiments. This attribute of the root symbionts in the present context of environment pollution can be expolited and utilised for agricultural benefits.

Fly ash

Soil amendment with fly ash of thermal power plant origin influenced plant growth and yield of black gram. Fly ash levels upto 40% were generally beneficial for black gram growth, above which it had a deleterious effect. The beneficial effects of fly ash added to the soil were optimal at 20%. In some earlier works promotion of plant

growth and yield at lower levels of fly ash has been reported (Plank, *et. al*, 1975; Singh, 1989; Pasha 1991; Jahan, 1993). The fly ash of Kasimpur thermal power plant origin have been found to contain some utilizable nutrients like K, Zn, B, Mn etc (Pasha, 1991). These nutrients may have increased plant growth and yield of black gram. Druzina *et. al.* (1982) also attributed enhanced plant growth and yield to utilizable plant nutrients available in fly ash. The nutrients from fly ash have been reported beneficial to plants through soil application or foilar dusting (Mishra and Shkula, 1984). Fly ash increases ion exchange capicity, water holding capacity and porosity of the soil. It also neutralises soil to some extent because of its highly alkaline nature (Jones and Straughan, 1978; Adriano *et al.*, 1980; Elseewi *et al.*, 1978). These favourable effects of fly ash in soil may have also contributed to the improved plant growth and yield of black gram. The gradual decline in measured growth and yield parameters above 40% fly ash was probably due to salinity caused by higher levels of sulphate, chloride, carbonate and bicarbonate in the amemded soil. These salts are reported to be much higher in higher concentration of fly ash (Pasha, 1991). Excessive uptake of elements and subsequent accumulation in the plants may have caused reduced growth and yield of black gram. Accumulation of boron is reported to cause suppressive effect on tomato (Francois, 1984), french bean and rhodes grass (Aitken and Bell, 1985). Some other toxic compounds like dibenzofuran, dibenzo-p-dioxine mixture (Helder *et al.*, 1982; Sawyer *et al.*, 1983) and metals like Ni, Ar, Cd, Cr, Pb, Se, Zn, Cu etc. (Wong and Wong 1986; Wadge and Hutton, 1987) reported to occur in fly ash, may have contributed towards poor growth and yield of black gram plants at higher levels of fly ash. Phytotoxicity of these metals has been established on some crop plants (Mayer, 1981; Hale *et al.*, 1985). The concentration of these compounds and metals at higher levels of fly ash may have exceeded the tolerance level of black gram. At lower levels the beneficial effect of fly ash may have masked their negative effects. Fly ash lacks nitrogen and its application to soil particularly at higher levels might have resulted into severe deficiency of nitrogen in soil as well as in plant tissue, which may also have suppressed growth and yield of black gram.

Favourable and harmful effects of fly ash at lower and higher levels respectively were found on leaf chlorophyll and seed protein. A

positive correlation has been established between leaf pigments and seed proteins with plant growth (Singh,1988; Pasha,1991; Jahan, 1993). It implies that plants due to improved soil nutrients and alteration in physico-chemical characters of soil as a result of fly ash application at lower levels synthesize more leaf pigments and proteins but their synthesis was suppressed at higher levels . Fly ash enhanced the both determined parameters of root nodulation of black gram at lower levels and suppressed at higher levels. Highest inhibition occurred at 100% level. Heavy metals and other toxic materials present in the fly ash may have suppressed root nodulation. Soil alkalinity also inhibits the activity of bacteria. Increased alkalinity due to application of fly ash may have inhibited root nodule bacteria, resulting in significant suppression of root nodulation.

Root colonization and spore production by *G.caledonicum* were influenced as a result of fly ash application to soil. Root colonization showed stepwise suppression with an increase in fly ash level in soil while spore production showed initial increase (upto 40% level) and then after declined. Infection of plant root by VAM fungi is inhibited by heavy metals. The infection of onion with *Glomus mosseae* was greatly reduced in the presence of Zn, Cu, Ni or Cd in the soil medium (Glidon and Tinker, 1983 a,1983 b). Population of VAM fungi was found to be reduced at a site polluted with heavy metals like Cd, Pb, Zn (letswaart *et al.*, 1992). Jahan (1993) also suggested that suppression of root colonization of chick pea and mungbean was due to the presence of heavy metals and other toxic compounds in fly ash.

At lower levels of fly ash, phosphorus content of black gram was found to be increased. Increased soil fertility decreases mycorrhizal development (Daft and Nicolson, 1972; Hayman and Mosse, 1972; Khan, 1975). It is established that high concentration of P in soil retards mycorrhizal developement (Mosse, 1991) because of dependency of the plants on the fungal associations is decreased (Ojala *et al.*, 1983).

Poor growth of root may have also provided reduced root surface area for development of nodules and for colonization of the VAM fungus. The two symbionts may have entered also into competition for root space leading to mutual adverse effects. Toxic effects of higher levels of

fly ash was reduced by the presence of the two root symbionts *Rhizobium* sp. and *G. caledonicum* on black gram. This was evident from better plant growth and yield and higher leaf chlorophyll, seed protein and N and P contents in dual inoculated plants than the uninoculated and single inoculated plants even at higher levels of fly ash. The root nodule bacterium and VAM fungus, therefore, provided protection to black gram plants to some extent against the adverse effect of fly ash at higher levels. This attribute is of immense agricultural value and artificial inoculation of legumes with these two root symbionts, can benefit farmers for growing agricultural crop around coal-fired thermal power plants, where soils suffer from gradual accumulation of fly ash. The present study shows that fly ash at lower concentration can ameliorate plant growth and yield and it can be used as a supplement to fertilizers. This would help in reducing cost of production and management of this waste. In most countries fly ash as a fertilizers is not commercially used for production. Fly ash is being used for manufacturing cement, buildings blocks but still large portion of the ash requires other eco-friendly uses. Use of fly ash to improve crop production can be an important management measure of this wastes which would also decrease total dependency on chemical fertilizers.

SUMMARY

In this study effects of two gaseous air pollutants (SO_2 and O_3) and one particulate air pollutant (fly ash) on plant growth and yield parameters,leaf cholorophyll,seed protein,root colonization and spore production by a VAM fungus, (*Glomus caledonicum*), root nodulation by *Rhizobium* sp., phosphrous and nitrogen contents of black gram plants were determined under artificial treatment conditions. Artifically generated SO_2 and O_3 were used for exposure of black gram plants grown in pots and fly ash of a coal-fired thermal power plant origin was incorporated in soil for the study. Root symbionts used in the study were a root nodule bacterium ,*Rhizobium* sp. and a VAM fungus,*Glomus caledonicum*. Two different concentrations of SO_2 (0.05 and 0.1 ppm) and O_3 (0.02-0.05-0.02 ppm and 0.05-0.1-0.05 ppm) were used for the exposure of the plants.Exposures were made in exposure chambers for 3h (SO_2) and 7h (O_3) respectively on alternate days. Fly ash was added to the sandy loam field soil to achieve its percentage in soil as 20,40,60,80,100.

Effect of sulphur dioxide

Inoculation of the black gram plants with in the root symbionts singly or in combination resulted in enhanced plant growth,yield,leaf chlorophyll,seed protein and P and N contents,in comparison to non-symbiotic (uninoculated) plants.This effect was greatest in dual inoculated plants.

Plant growth parameters (length and fresh and dry weights) of black gram were significantly reduced at both the concentrations of SO_2.Reductions were greater at 0.1 ppm than at 0.05 ppm of SO_2. Plants inoculated with both root symbionts (dual inoculation) and exposed to SO_2 had greater lengths, fresh and dry weights of shoots and roots than uninoculated unexposed plants. The yield of the plants determined on the basis of pod number/plant and seed number /pod was also reduced but the reductions at both the concentrations were not significant. Chlorophyll content (chlorophyll a,b and total chlorophyll) of leaves was significantly reduced at both the concentrations.Dual inoculated exposed plants,however,contained greater amount of chlorophyll than uninoculated unexposed plants.Seed protein was also adversely affected by the SO_2

concentrations. Highest reduction occurred at 0.1 ppm. Exposed plants inoculated with the root symbionts (single either with *Rhizobium* sp. or *G. caledonicum*) or in combination (*Rhizobium* sp. + *G. caledonicum*) also showed greater seed protein compared to uninoculated unexposed plants.

Root colonization and spore productions by the VAM fungus on black gram was suppressed by exposure of the plants to SO_2 concentrations.Root colonization and spore production on dual inoculated plants were significantly greater than plants inoculated with the VAM fungus alone. Root and shoot of nodulated (inoculated with *Rhizobium* sp. alone),mycorrhizal (inoculated with *G. caledonicum* alone) and dual inoculated plants exposed to SO_2 concentrations (0.05 and 0.1 ppm.) showed significantly reduced phosphorus contents.Phosphorus content of root and shoot of dual inoculated exposed plants was highest among these three treatments.

Root nodulation (number of nodules /root system and dry weight of nodules) of black gram plants was significantly inhibited in exposed treatments of inoculated and uninoculated plants compared to their respective unexposed controls. Reductions were greater at 0.1 ppm SO_2.At both the concentrations of SO_2, a significant reduction occurred in root nodulation of dual inoculated plants,compared to unexposed dual inoculated plants. Nitrogen content of shoot and root also showed a significant decrease at both the concentrations of SO_2. Shoot and root of uninoculated plants contained significantly reduced nitrogen at both the concentration of SO_2 in comparison to uninoculated unexposed black gram plants. Nitrogen contents of shoot and root of exposed dual inoculated plants was higher than the uninoculated unexposed plants.In overall assessment, SO_2 exposure suppressed plant growth and other considered parameters of black gram except yield which did not significantly decline.Growth performance and yield of the plants were greatly improve by the root symbionts.Dual inoculated plants performed best in this respect. In the presence of root symbionts,particularly in dual inoculated plants,adverse effect of SO_2 exposure was less.Root nodulation by *Rhizobium* sp.and root colonization by *G. caledonicum* were also suppressed.But even in suppressed state,the root symbionts were able to provide partial protection to the plants from adverse effects of SO_2.

Effect of ozone

Like SO_2 experiment,plant growth parameters (length,fresh and dry weights of shoot and root) were greater in dual inoculated plants than plants inoculated with either of the root symbionts.O_3 was also suppressive for growth and yield of black gram plants.The root and shoot lengths were significantly reduced . Lengths, fresh and dry weights of root and shoot were significantly reduced in both the treatments of O_3 (0.02-0.05-0.02 and 0.05-0.1-0.05 ppm) as compared to controls, except in dual inoculated plants in which reduction in shoot and root lenghts at 0.02-0.05-0.02 ppm were not significant. Yield of single and dual inoculated and uninoculated plants of black gram was also significantly affected by O_3 treatments in comparison to their respective unexposed controls.O_3 reduced pod number and number of seeds per pod significantly. Leaf chlorophyll (chlorophyll a, chlorophyll b and total chlorophyll) of inoculated and uninoculated plants exposed to O_3 treatments was significantly reduced in both concentration of O_3, compared with their respective controls. Seed protein was also effected adversely by O_3. Seed protein of uninoculated, nodulated, mycorrhizal and dual inoculated plants exposed to both concentrations of O_3 was signifcantly reduced in comparison to their respective controls. Nodulated, mycorrhizal and dual inoculated plants had better plant growth, yield, leaf chlorophyll, seed protein and P and N contents in comparison to uninoculated plants. This effect was greatest in dual inoculated plants. Single or dual inoculated plants exposed to O_3 concentrations also showed higher values of these parameters in comparison to uninoculated exposed plants.

Root colonization and spore production by the VAM fungus *G.caledonicum* on black gram decreased by exposure of the plants to O_3 concentrations. Dual inoculated plants showed greater root colonization and spore production than plants inoculated with the VAM fungus alone. Phosphorus content of shoots and roots of nodulated, mycorrhizal and dual inoculated plants exposed to O_3 concentrations significantly declined. Dual inoculated exposed plants showed greater phosphorus content than mycorrhizal and nodulated plants.

Root nodulation of both inoculated and uninoculated black gram

plants was significantly suppressed by exposure to O_3 concentrations. Nodule number and dry weight of nodules of dual inoculated and nodulated plants significantly declined. Shoot and root nitrogen also showed a significant decrease at both the concentrations of O_3. Exposed uninoculated black gram plants showed a significant reduction in nitrogen content of shoot and root. Dual inoculated exposed plants had highest root and shoot nitrogen. In general, O_3 exposures suppressed plant growth, yield and other considered parameters of black gram. Though plant growth and yield were greatly improved by the root symbionts, with dual inoculated plants performing best, O_3 also inhibited the root symbionts to a varying extents. Adverse effects of O_3 exposures was, however, less in the presence of the root symbionts,particularly in dual inoculated plants. The root symbionts though suppressed by O_3, provided partial protection to the plants from adverse effects of O_3.

Effect of fly ash

Plant growth and yield of black gram plants were enhanced by soil amendment with fly ash, which was related to the level of fly ash in soil. At low levels, beneficial effects of fly ash was optimal at 40% and plant growth and yield increased.

Shoot length of uninoculated and nodulated plants was greater than control at 20, 40 and 60% levels of fly ash but in mycorrhizal and dual inoculated plants, no significant increase occurred at 20 and 40% levels. At 60% levels shoot lengths declined. Root length in uninoculated, nodulated and mycorrhizal plants significantly increased, as compared to their respective controls upto 60% level. In dual inoculated plants, this increase was only upto 20%, with a decline at 40% onwards. Fresh weight of shoot increased significantly upto 40% in most of treatments, but root fresh weight showed a decrease. At 60% level shoot fresh weight was not significant in most of the treatments compared to their respective controls. Fly ash at 80% and 100% levels caused a significant decline in plant growth in most of the treatments. Dry weight of root and shoot increased upto 60% level of fly ash in uninoculated, mycorrhizal and nodulated plants in comparison to their respective controls. Dual inoculated plants showed an increase only in

shoot. Yield of black gram significantly increased from 20 to 60% levels of fly ash in all the treatments. At 80 and 100% levels significant reduction in yield occurred. Chlorophyll content also showed an increase upto 40% level. At 60% level significant decrease occurred in chlorophyll a in inoculated plants. Significant reduction in chlorophyll b and total chlorophyll occurred at 60% level in all the treatments. Further reduction occurred in chlorophyll content at 80 and 100% levels of fly ash. Seed protein was also greater at 20 and 40% levels in all the treatments. But at 60% level, seed protein declined in uninoculated and mycorrhizal plants with exception of nodulated and dual inoculated plants in comparison to their respective controls.

Root colonization decreased at all levels of fly ash in dual (*Rhizobium* sp.+ *G.caledonicum*) and single (*G.caledonicum*) inoculated plants. Spore production by the VAM fungus and phosphorus contents of shoot and root significantly increased at low levels (20-40%) of fly ash. At 60% levels, spore production, phosphorus contents of shoot and root decreased in most of the treatments except phosphorus content of shoot in dual inoculated plants. At 80% and 100% levels significant reduction occurred in spore production and phosphorus content. Significant increase occurred in nodulation, nitrogen content of shoot and root at 20% and 40% levels of fly ash. From 60% onwards, reductions in root nodulation and nitrogen content were observed. Fly ash ,in general ,was therefore beneficial at lower levels for black gram with respect to all considered parameters.Adverse effects of fly ash occurred at higher levels .

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ANOVA TABLES

Table 1a. Effect of SO_2 on shoot length of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	16.286	3.257	0.1048
Treatment	2	225.255	112.627	199.453
SO_2	3	428.348	142.782	252.856
Interaction	6	19.8933	3.3155	5.8715
Error	55	31.057	0.5646	

Table 1b. Effect of SO_2 on root length of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	4.1208	0.8241	0.0208
Treatment	2	169.259	84.629	118.032
SO_2	3	399.007	133.002	185.496
Interaction	6	100.2111	16.7018	23.293
Error	55	39.435	0.7170	

Table 1c. Effect of SO_2 on fresh weight of shoot of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.29922	0.05984	0.02246
Treatment	2	73.1136	36.556	754.6959
SO_2	3	201.563	67.187	1387.05
Interaction	6	3.5635	0.5939	12.2613
Error	55	2.6641	0.0484	

Table 1d. Effect of SO_2 on fresh weight of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.14641	0.02928	0.01733
Treatment	2	12.1129	6.0564	197.226
SO_2	3	3.140	1.0467	34.087
Interaction	6	1.9024	0.31707	10.3252
Error	55	1.6889	0.030708	

Table 2a. Effect of SO_2 on dry weight of shoot of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.041304	0.00826	0.01713
Treatment	2	76.6041	38.3020	4368.81
SO_2	3	88.3140	29.438	3357.763
Interaction	6	6.66157	1.11026	126.638
Error	55	0.48219	0.008767	

Table 2b. Effect of SO_2 on dry weight of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.01187	0.00237	0.02400
Treatment	2	4.779558.	2.3977	1332.838
SO_2	3	56.481	18.827	10465.198
Interaction	6	14.1447	2.357	1310.4177
Error	55	0.0989	0.00179	

Table 2c. Effect of SO_2 on pod number of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	19.508	3.9016	0.01803
Treatment	2	92.572	46.286	11.7699
SO_2	3	122.421	40.807	10.376
Interaction	6	6.3977	1.0662	0.27114
Error	55	216.219	3.9325	

Table 2d. Effect of SO_2 on seed number/pod of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	753.9232	150.7846	5.52124
Treatment	2	3984.366	1992.183	72.9473
SO_2	3	13567.040	4522.346	165.5983
Interaction	6	648.0198	108.0033	3.95473
Error	55	1502.044	27.3098	

Table 3a. Effect of SO_2 on chlorophyll a of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.000002742	0.000000548	0.0226
Treatment	2	0.0000341	0.000017073	38.8399
SO_2	3	0.000446	0.0001488	338.581
Interaction	6	0.0000951	0.00001586	36.0936
Error	55	0.0000241	0.000000440	

Table 3b. Effect of SO_2 on chlorophyll b of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.00000259	0.000000519	0.00507
Treatment	2	0.001609	0.0008049	4329711
SO_2	3	0.0378	0.012633	6795.545
Interaction	6	0.000432	0.00007216	38.818
Error	55	0.0001022	0.000001859	

Table 3c. Effect of SO_2 on total chlorophyll of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.0000198	0.0000396	0.03771
Treatment	2	0.001848	0.000924	483.720
SO_2	3	0.03019	0.01006	52.364
Interaction	6	0.000480	0.00008008	41.904
Error	55	0.0001051	0.00000191	

Table3d. Effect of SO_2 on seed protein of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	25.86	5.1720	0.0832
Treatment	2	22.348	11.1741	9.8970
SO_2	3	151.867	50.622	44.83
Interaction	6	13.944	2.324	2.058
Error	55	62.096	1.1290	

Table 4a. Effect of SO_2 on spore number of VAM fungus of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	10.125	2.025	0.0111
Treatment	2	92596.0	46298	14000.76
SO_2	3	1660104	553368	167341.51
Interaction	6	130188.00	21698	6561.594
Error	55	181.875	3.30681	

Table 4b. Effect of SO_2 on root colonization by VAM of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	11.714	2.3429	0.01753
Treatment	2	34.23	17.115	7.0449
SO_2	3	105230.42	35076.80	14438.44
Interaction	6	36.679	6.1132	2.516
Error	55	133.617	2.4292	

Table 4c. Effect of SO_2 on phosphorus content of shoot of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.0000423	0.00000847	0.04885
Treatment	2	0.01620	0.008102	2570.182
SO_2	3	0.5356	0.17855	56638.183
Interaction	6	0.00157	0.000261	83.0041
Error	55	0.0001733	0.00000315	

Table 4d. Effect of SO_2 on phosphorus content of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.0000133	0.000002677	0.01739
Treatment	2	0.018400	0.009204	3289.971
SO_2	3	0.1478	0.04928	17617.9707
Interaction	6	0.00793	0.00132	472.638
Error	55	0.000153	0.00000279	

Table 5a. Effect of SO_2 on nodule number of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	9.444	1.888	0.039
Treatment	2	24.111	12.055	13.942
SO_2	3	1200.05	400.01	462.63
Interaction	6	24.777	4.129	4.7761
Error	55	47.555	0.8646	

Table 5b. Effect of SO_2 on nodule dry weight of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.00204	0.000408	0.01714
Treatment	2	35.111	17.555	40563.58
SO_2	3	1493.75	497.919	1150475.375
Interaction	6	35.86	5.977	13812.098
Error	55	0.0238	0.000432	

Table 5c. Effect of SO_2 on nitrogen content of shoot of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.00143	0.000287	0.02471
Treatment	2	0.0042	0.00212	10.027
SO_2	3	0.0528	0.0176	83.165
Interaction	6	0.0068	0.00114	5.424
Error	55	0.01164	0.000211	

Table 5d. Effect of SO_2 on nitrogen content of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.0000214	0.000004294	0.02636
Treatment	2	0.00167	0.000838	283.104
SO_2	3	0.0348	0.01161	3924.54
Interaction	6	0.000638	0.000106	35.923
Error	55	0.000162	0.00000296	

Table 6a. Effect of O_3 on shoot length of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	3.479	o.6958	0.22748
Treatment	2	571.750	285.87	93.461
O_3	3	578.479	192.82	63.0407
Interaction	6	56.739	9.4565	3.0916
Error	55	168.231	13.0587	

Table 6b. Effect of O_3 on root length of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	11.419	2.283	1.1905
Treatment	2	87.619	43.809	22.837
O_3	3	447.78	149.262	77.807
Interaction	6	71.631	11.9138	6.223
Error	55	105.50193	1.9183	

Table 6c. Effect of O_3 on fresh weight of shoot of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.86523	.17304	1.0290
Treatment	2	330.55	165.2765	982.797
O_3	3	385.718	128.57	764.54
Interaction	6	10.9729	1.8288	10.874
Error	55	9.249	0.16816	

Table 6d. Effect of O_3 on fresh weight of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.008931	0.001786	2.3305
Treatment	2	48.901	24.450	31901.370
O_3	3	212.809	70.936	92551.63
Interaction	6	47.329	7.888	10291.96
Error	55	0.04215	0.000766	

Table 7a. Effect of O_3 on dry weight of shoot of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.035644	0.007128	0.77824
Treatment	2	7.7192	3.8596	421.3457
O_3	3	55.1137	18.3712	2005.53
Interaction	6	0.40289	0.06714	7.3305
Error	55	0.50381	0.0091602	

Table 7b. Effect of O_3 on dry weight of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.000381	0.0000762	2.1421
Treatment	2	0.563939	0.31969	8976.442
O_3	3	21.746	7.248	203535.400
Interaction	6	0.2539	0.04232	1188.37
Error	55	0.001958	0.0000356	

Table 7c. Effect of O_3 on pod number of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	27.590	5.518	3.939
Treatment	2	40.393	20.196	14.418
O_3	3	119.511	39.83	28.439
Interaction	6	19.335	3.222	2.3006
Error	55	77.04	1.400	

Table 7d. Effect of O_3 on seed number/pod of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	30.2239	6.0447	0.5916414
Treatment	2	4442.1770	2221.089	217.391
O_3	3	17777.030	5925.675	579.982
Interaction	6	434.756	72.459	7.09205
Error	55	561.934	10.21699	

Table 8a. Effect of O_3 on chlorophyll a of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.000364	0.00007298	2.441
Treatment	2	0.22400	0.112004	3746.87
O_3	3	1.02712	0.34237	11453.51
Interaction	6	0.11573	0.019288	645.272
Error	55	0.001644	0.0000298	

Table 8b. Effect of O_3 on chlorophyll b of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.00001589	0.000003179	0.906095
Treatment	2	0.25001	0.12500	35630.670
O_3	3	1.3991	0.46639	132937.100
Interaction	6	0.220947	0.03682	10496.220
Error	55	0.0001929	0.00000350	

Table 8c. Effect of O_3 on total chlorophyll of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.1611176	0.32223	0.963194
Treatment	2	1.52401	0.762006	22.7771
O_3	3	5.00640	1.6688	49.882
Interaction	6	0.89853	0.14975	4.47633
Error	55	1.84001	0.033454	

Table 8d. Effect of O_3 on seed protein of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.0091145	0.001822	6.1599
Treatment	2	162.863	81.4316	275173.800
O_3	3	158.806	52.935	178879.100
Interaction	6	11.256	1.8760	6339.669
Error	55	0.01627	0.000295	

Table 9a. Effect of O_3 on spore number of VAM fungus of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	18.6250	3.72500	2.15799
Treatment	2	7447.792	3723.896	2157.359
O_3	3	597565.1	199188.400	115395.50
Interaction	6	7463.33	1243.889	720.6203
Error	55	94.93750	1.726130	

Table 9b. Effect of O_3 on root colonization by VAM of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	3.8802	0.77604	0.851871
Treatment	2	196.5052	98.252	107.853
O_3	3	78431.38	26143.790	28698.380
Interaction	6	201.627	33.6046	36.88821
Error	55	50.1041	0.9109	

Table 9c. Effect of O_3 on phosphorus content of shoot of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.005877	0.001175	0.881161
Treatment	2	0.19990	0.09995	74.9301
O_3	3	4.87162	1.62387	1217.333
Interaction	6	0.16919	0.02819	21.13988
Error	55	0.07336	0.00133	

Table 9d. Effect of O_3 on phosphorus content of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.00001887	0.00000377	1.098
Treatment	2	0.00811	0.004056	1180.43
O_3	3	0.02986	0.009953	2896.688
Interaction	6	0.000508	0.0000846	24.6403
Error	55	0.000188	0.00000343	

Table 10a. Effect of O_3 on nodule number of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	1.2343	0.24686	0.22049
Treatment	2	22.1124	11.05620	9.8751
O_3	3	656.128	218.709	195.347
Interaction	6	23.457	3.9095	3.4919
Error	55	61.57	1.11959	

Table 10b. Effect of O_3 on nodule dry weight of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	4.542	0.90840	1.0237
Treatment	2	30.0025	15.0012	16.9068
O_3	3	1091.341	363.780	409.989
Interaction	6	30.176	5.0293	5.6682
Error	55	48.801	0.88729	

Table10c. Effect of O_3 on nitrogen content of shoot black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.007019	0.001403	0.98024
Treatment	2	2.7655	1.38275	965.540
O_3	3	23.736	7.91231	5524.944
Interaction	6	0.15289	0.025481	17.7931
Error	55	0.7876	0.001432	

Table 10d. Effect of O_3 on nitrogen content of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.000224	0.0000448	0.57402
Treatment	2	0.201971	0.100985	12911.453
O_3	3	2.1294	0.70980	9077.318
Interaction	6	0.16722	0.02787	356.424
Error	55	0.0004300	0.0000781	

Table 11a. Effect of soil amendment with fly ash on length of shoot of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	10.64	2.1288	0.00810
Treatment	5	1604.78	320.95	140.511
Fly ash	3	2072.19	690.730	302.3935
Interaction	15	273.35	18.22	7.97
Error	115	262.698	2.284	

Table 11b. Effect of soil amendment with fly ash on length of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	15.745	3.149	0.0371
Treatment	5	162.88	32.57	44.177
Fly ash	3	760.106	253.36	343.58
Interaction	15	85.09	5.672	7.692
Error	115	84.80	0.7374	

Table 12a. Effect of soil amendment with fly ash on fresh weight of shoot of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	13.512	2.702	0.04160
Treatment	5	272.18	54.43	96.364
Fly ash	3	197.44	65.814	116.504
Interaction	15	18.96	1.264	2.238
Error	115	64.96	0.5649	

Table 12b. Effect of soil amendment with fly ash on fresh weight of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	1.323	0.264	0.0252
Treatment	5	15.270	3.054	33.497
Fly ash	3	91.451	30.483	334.34
Interaction	15	8.31	0.554	6.084
Error	115	10.485	0.0911	

Table 13a. Effect of soil amendment with fly ash on dry weight of shoot of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.0351	0.007039	0.00657
Treatment	5	208.76	41.752	4484.206
Fly ash	3	99.991	33.33	3579.67
Interaction	15	14.989	0.999	107.321
Error	115	1.070	0.0093	

Table 13b. Effect of soil amendment with fly ash on dry weight of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.00376	0.000752	0.00498
Treatment	5	7.020	1.14040	1068.33
Fly ash	3	17.92	5.975	4546.56
Interaction	15	13.67	0.9114	693.493
Error	115	0.1511	0.00131	

Table 14a. Effect of soil amendment with fly ash on pod number /plant of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	7.383	1.476	0.01077
Treatment	5	972.42	194.485	163.2640
Fly ash	3	327.47	109.159	91.635
Interaction	15	72.72	4.848	4.0697
Error	115	136.99	1.1912	

Table 14b. Effect of soil amendment with fly ash on seed number/pod of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	240.724	48.144799	1.465909
Treatment	5	54453.140	10890.6300	331.59710
Fly ash	3	27025.1400	9008.3800	274.2865
Interaction	15	4706.695	313.77970	9.55394
Error	115	3776.9400	32.84296	

Table 15a. Effect of soil amendment with fly ash on chlorophyll a of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.00001847	1.00000369	0.01189
Treatment	5	0.001887	0.0003774	139.7877
Fly ash	3	0.000838	0.000279	103.468
Interaction	15	0.000120	0.000008005	2.964
Error	115	0.00031	0.00000270	

Table 15b. Effect of soil amendment with fly ash on chlorophyll b of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.04212	0.00842	0.00840
Treatment	5	0.0887	0.01775	2.0378
Fly ash	3	0.0841	0.02806	3.2207
Interaction	15	0.3380	0.0225	2.586
Error	115	1.0020	0.00871	

Table 15c. Effect of soil amendment with fly ash on total chlorophyll of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.00004010	0.00000802	0.01791
Treatment	5	0.4588	0.09176	23572.55
Fly ash	3	0.04222	0.01407	3615.412
Interaction	15	0.01460	0.0009736	250.092
Error	115	0.000447	0.0000389	

Table 16. Effect of soil amendment with fly ash on seed protein of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.00004010	0.00000802	0.01791
Treatment	5	0.4588	0.09176	23572.55
Fly ash	3	0.04222	0.01407	3615.412
Interaction	15	0.01460	0.0009736	250.092
Error	115	0.000447	0.00000389	

Table 17a. Effect of soil amendment with fly ash on spore number of VAM fungus of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	18.652	3.7305	0.0304
Treatment	5	1416.81	283.36	266.397
Fly ash	3	835.11	278.371	261.704
Interaction	15	1230.11	82.007	77.097
Error	115	122.32	1.063	

Table 17b. Effect of soil amendment with fly ash on root colonization by VAM of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	5.1794	1.0358	0.009510
Treatment	5	2692.968	538.5936	568.648
Fly ash	3	127383.85	42461.28	44830.738
Interaction	15	2965.61	197.707	208.740
Error	115	108.921	0.94714	

Table 18a. Effect of soil amendment with fly ash on phosphorus content of shoot of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.00117	0.0002345	0.01131
Treatment	5	0.02611	0.005223	28.970
Fly ash	3	0.0155	0.051760	28.710
Interaction	15	0.00717	0.000478	2.654
Error	115	0.0207	0.0001802	

Table 18b. Effect of soil amendment with fly ash on phosphorus content of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.00001576	0.00000315	0.00763
Treatment	5	0.001433	0.002866	797.826
Fly ash	3	0.02738	0.009128	2540.892
Interaction	15	0.001902	0.000126	35.3004
Error	115	0.000413	0.00000359	

Table 19a. Effect of soil amendment with fly ash on nodule number of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	2.4721	0.4944	0.00864
Treatment	5	201.722	40.344	81.1197
Fly ash	3	1726.69	575.564	1157.27
Interaction	15	207.222	13.8148	27.777
Error	115	57.194	0.4973	

Table 19b. Effect of soil amendment with fly ash on nodule dry weight of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	2.1546	0.43093	0.00706
Treatment	5	307.22	61.445	115.916
Fly ash	3	2805.70	93523	1764.307
Interaction	15	338.36	22557	42.554
Error	115	60.95	0.53008	

Table 20a. Effect of soil amendment with fly ash on nitrogen content of shoot of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.0001924	0.0000384	0.00523
Treatment	5	0.04772	0.009544	149.181
Fly ash	3	0.04777	0.01592	248.897
Interaction	15	0.00827	0.000551	8.619
Error	115	0.00735	0.00006398	

Table 20b. Effect of soil amendment with fly ash on nitrogen content of root of black gram plants

Sources	d.F.	TotalS.S	M.S.S	F.cal.
Replicates	5	0.02686	0.0053799	0.00523
Treatment	5	0.0382	0.00765	1.3956
Fly ash	3	0.04591	0.015305	2.7909
Interaction	15	0.09794	0.006529	1.19066
Error	115	0.63065	0.005483	